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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

 FILE 'HOME' ENTERED AT 15:38:17 ON 01 MAY 2003

 => fil .bec
 COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                            ENTRY    SESSION
 FULL ESTIMATED COST                                         0.21       0.21

 FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
        ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 15:38:34 ON 01 MAY 2003
 ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

 11 FILES IN THE FILE LIST

 => s esterase#
 FILE 'MEDLINE'
 L1        21683 ESTERASE#

 FILE 'SCISEARCH'
 L2        13004 ESTERASE#

 FILE 'LIFESCI'
 L3         5517 ESTERASE#

 FILE 'BIOTECHDS'
 L4         2068 ESTERASE#

 FILE 'BIOSIS'
 L5        43845 ESTERASE#

 FILE 'EMBASE'
 L6        14021 ESTERASE#

 FILE 'HCAPLUS'
 L7        33054 ESTERASE#

 FILE 'NTIS'
 L8          298 ESTERASE#

 FILE 'ESBIOBASE'
 L9         3385 ESTERASE#

 FILE 'BIOTECHNO'
 L10        4445 ESTERASE#

 FILE 'WPIDS'
 L11        2231 ESTERASE#

 TOTAL FOR ALL FILES
 L12      143551 ESTERASE#

 => s ferulic or cinnamic or phenolic or coumaric or feruloyl or cinnamoyl or
 coumaroyl
 FILE 'MEDLINE'
             758 FERULIC
             859 CINNAMIC
            6655 PHENOLIC
            1072 COUMARIC
             187 FERULOYL
             260 CINNAMOYL
             215 COUMAROYL
 L13        8737 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                 MOYL OR COUMAROYL
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FILE 'SCISEARCH'
          1899 FERULIC
          2001 CINNAMIC
         18588 PHENOLIC
          1199 COUMARIC
           360 FERULOYL
           713 CINNAMOYL
           487 COUMAROYL
L14       22607 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                MOYL OR COUMAROYL

FILE 'LIFESCI'
           403 FERULIC
           326 CINNAMIC
          3297 PHENOLIC
           248 COUMARIC
            61 FERULOYL
            88 CINNAMOYL
            83 COUMAROYL
L15        3974 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                MOYL OR COUMAROYL

FILE 'BIOTECHDS'
           299 FERULIC
           271 CINNAMIC
          1334 PHENOLIC
           129 COUMARIC
            63 FERULOYL
            63 CINNAMOYL
            43 COUMAROYL
L16        1918 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                MOYL OR COUMAROYL

FILE 'BIOSIS'
          2634 FERULIC
          2451 CINNAMIC
         17205 PHENOLIC
          1999 COUMARIC
           484 FERULOYL
           630 CINNAMOYL
           624 COUMAROYL
L17       22224 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                MOYL OR COUMAROYL

FILE 'EMBASE'
           964 FERULIC
          1563 CINNAMIC
          7209 PHENOLIC
           753 COUMARIC
           151 FERULOYL
           281 CINNAMOYL
           176 COUMAROYL
L18        9956 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                MOYL OR COUMAROYL

FILE 'HCAPLUS'
          6041 FERULIC
         15208 CINNAMIC
        128136 PHENOLIC
          4746 COUMARIC
           647 FERULOYL
          3899 CINNAMOYL
           885 COUMAROYL
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L19      149936 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                MOYL OR COUMAROYL

FILE 'NTIS'
             9 FERULIC
            53 CINNAMIC
          1891 PHENOLIC
            11 COUMARIC
             1 FERULOYL
            12 CINNAMOYL
             1 COUMAROYL
L20       1955 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                MOYL OR COUMAROYL

FILE 'ESBIOBASE'
           656 FERULIC
           439 CINNAMIC
          4204 PHENOLIC
           414 COUMARIC
           182 FERULOYL
           159 CINNAMOYL
           191 COUMAROYL
L21       5334 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                MOYL OR COUMAROYL

FILE 'BIOTECHNO'
           451 FERULIC
           361 CINNAMIC
          2742 PHENOLIC
           351 COUMARIC
           107 FERULOYL
           102 CINNAMOYL
            89 COUMAROYL
L22       3576 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                MOYL OR COUMAROYL

FILE 'WPIDS'
           326 FERULIC
          2275 CINNAMIC
         28118 PHENOLIC
           102 COUMARIC
            17 FERULOYL
           714 CINNAMOYL
            16 COUMAROYL
L23      31342 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                MOYL OR COUMAROYL

TOTAL FOR ALL FILES
L24     261559 FERULIC OR CINNAMIC OR PHENOLIC OR COUMARIC OR FERULOYL OR CINNA
                MOYL OR COUMAROYL

=> s l24(3a)l12
FILE 'MEDLINE'
L25          66 L13(3A)L1

FILE 'SCISEARCH'
L26         151 L14(3A)L2

FILE 'LIFESCI'
L27          59 L15(3A)L3

FILE 'BIOTECHDS'
L28          50 L16(3A)L4
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FILE 'BIOSIS'
L29         152 L17(3A)L5

FILE 'EMBASE'
L30          76 L18(3A)L6

FILE 'HCAPLUS'
L31         210 L19(3A)L7

FILE 'NTIS'
L32           0 L20(3A)L8

FILE 'ESBIOBASE'
L33          76 L21(3A)L9

FILE 'BIOTECHNO'
L34          81 L22(3A)L10

FILE 'WPIDS'
L35          10 L23(3A)L11

TOTAL FOR ALL FILES
L36         931 L24(3A) L12

=> s l36 not 1998-2000/py
FILE 'MEDLINE'
       1384733 1998-2000/PY
L37          45 L25 NOT 1998-2000/PY

FILE 'SCISEARCH'
       2912462 1998-2000/PY
L38         108 L26 NOT 1998-2000/PY

FILE 'LIFESCI'
        334799 1998-2000/PY
L39          43 L27 NOT 1998-2000/PY

FILE 'BIOTECHDS'
         42739 1998-2000/PY
L40          34 L28 NOT 1998-2000/PY

FILE 'BIOSIS'
       1689852 1998-2000/PY
L41         110 L29 NOT 1998-2000/PY

FILE 'EMBASE'
       1302504 1998-2000/PY
L42          53 L30 NOT 1998-2000/PY

FILE 'HCAPLUS'
       2674369 1998-2000/PY
L43         147 L31 NOT 1998-2000/PY

FILE 'NTIS'
         73541 1998-2000/PY
L44           0 L32 NOT 1998-2000/PY

FILE 'ESBIOBASE'
        853099 1998-2000/PY
L45          42 L33 NOT 1998-2000/PY

FILE 'BIOTECHNO'
        355338 1998-2000/PY
L46          52 L34 NOT 1998-2000/PY
```

```
FILE 'WPIDS'
       2478567 1998-2000/PY
L47           2 L35 NOT 1998-2000/PY

TOTAL FOR ALL FILES
L48         636 L36 NOT 1998-2000/PY

=> s l48 not 2001-2003/py
FILE 'MEDLINE'
       1186051 2001-2003/PY
L49          26 L37 NOT 2001-2003/PY

FILE 'SCISEARCH'
       2162491 2001-2003/PY
L50          68 L38 NOT 2001-2003/PY

FILE 'LIFESCI'
        197014 2001-2003/PY
L51          32 L39 NOT 2001-2003/PY

FILE 'BIOTECHDS'
         41958 2001-2003/PY
L52          26 L40 NOT 2001-2003/PY

FILE 'BIOSIS'
       1152615 2001-2003/PY
L53          65 L41 NOT 2001-2003/PY

FILE 'EMBASE'
        979363 2001-2003/PY
L54          32 L42 NOT 2001-2003/PY

FILE 'HCAPLUS'
       2281920 2001-2003/PY
L55          94 L43 NOT 2001-2003/PY

FILE 'NTIS'
         31124 2001-2003/PY
L56           0 L44 NOT 2001-2003/PY

FILE 'ESBIOBASE'
        631406 2001-2003/PY
L57          20 L45 NOT 2001-2003/PY

FILE 'BIOTECHNO'
        255963 2001-2003/PY
L58          33 L46 NOT 2001-2003/PY

FILE 'WPIDS'
       2123015 2001-2003/PY
L59           1 L47 NOT 2001-2003/PY

TOTAL FOR ALL FILES
L60         397 L48 NOT 2001-2003/PY

=> dup rem l60
PROCESSING COMPLETED FOR L60
L61          135 DUP REM L60 (262 DUPLICATES REMOVED)

=> d tot

L61  ANSWER 1 OF 135  HCAPLUS  COPYRIGHT 2003 ACS
TI   Hydrolases involving lignocellulose degradation from various
```

micro-organisms (hydroxycinnamyl **esterase**, **feruloyl esterases**)
SO (1997) 175 pp. Avail.: UMI, Order No. DA9728563
From: Diss. Abstr. Int., B 1997, 58(4), 1894
AU Zhao, Yongxin
AN 1997:662679 HCAPLUS
DN 127:293501

L61 ANSWER 2 OF 135 HCAPLUS COPYRIGHT 2003 ACS
TI Antitumoric phenolic acid sugar ester enzymic manufacture
SO Jpn. Kokai Tokkyo Koho, 11 pp.
CODEN: JKXXAF
IN Massuda, Kazuaki; Hagiwara, Toshihiko; Ishikaki, Eishi; Kaneko, Hiroaki; Kikuta, Keitaro; Aoki, Hitoshi
AN 1997:809845 HCAPLUS
DN 128:101159

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 09322794	A2	19971216	JP 1997-43960	19970227

L61 ANSWER 3 OF 135 MEDLINE DUPLICATE 1
TI The faeA genes from Aspergillus niger and Aspergillus tubingensis encode **ferulic** acid **esterases** involved in degradation of complex cell wall polysaccharides.
SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1997 Dec) 63 (12) 4638-44.
Journal code: 7605801. ISSN: 0099-2240.
AU de Vries R P; Michelsen B; Poulsen C H; Kroon P A; van den Heuvel R H; Faulds C B; Williamson G; van den Hombergh J P; Visser J
AN 1998069455 MEDLINE

L61 ANSWER 4 OF 135 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI The faeA genes from Aspergillus niger and Aspergillus tubingensis encode **ferulic**-acid-**esterases** involved in degradation of complex cell wall polysaccharides;
such as wheat arabinoxylan and sugar beet pectin
SO Appl.Environ.Microbiol.; (1997) 63, 12, 4638-44
CODEN: AEMIDF ISSN: 0099-2240
AU de Vries R P; Michelsen B; Poulsen C H; Kroon P A; van den Heuvel R H H; Faulds C B; Williamson G; van den Hombergh J P T W; *Visser J
AN 1998-01759 BIOTECHDS

L61 ANSWER 5 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Three Neocallimastix patriciarum esterases associated with the degradation of complex polysaccharides are members of a new family of hydrolases
SO MICROBIOLOGY-UK, (AUG 1997) Vol. 143, Part 8, pp. 2605-2614.
Publisher: SOC GENERAL MICROBIOLOGY, MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING, BERKS, ENGLAND RG7 1AE.
ISSN: 1350-0872.
AU Dalrymple B P (Reprint); Cybinski D H; Layton I; McSweeney C S; Xue G P; Swadling Y J; Lowry J B
AN 97:615530 SCISEARCH

L61 ANSWER 6 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Antioxidant activity of corn bran cell-wall fragments in the LDL oxidation system
SO JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, (MAY 1997) Vol. 45, No. 5, pp. 1644-1648.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.
ISSN: 0021-8561.
AU Ohta T (Reprint); Semboku N; Kuchii A; Egashira Y; Sanada H
AN 97:403289 SCISEARCH

L61 ANSWER 7 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 2
TI Chemical characterization and spectroscopic analysis of the solubilization

products from wheat straw produced by Streptomyces strains grown in solid-state fermentation
SO MICROBIOLOGY-UK, (APR 1997) Vol. 143, Part 4, pp. 1359-1367.
Publisher: SOC GENERAL MICROBIOLOGY, HARVEST HOUSE 62 LONDON ROAD, READING, BERKS, ENGLAND RG1 5AS.
ISSN: 1350-0872.
AU HernandezCoronado M J; Hernandez M; Centenera F; PerezLeblic M I; Ball A S; Arias M E (Reprint)
AN 97:344719 SCISEARCH

L61 ANSWER 8 OF 135 MEDLINE DUPLICATE 3
TI Purification and characterization of a **feruloyl esterase** from the fungus Penicillium expansum.
SO JOURNAL OF APPLIED MICROBIOLOGY, (1997 Dec) 83 (6) 718-26.
Journal code: 9706280. ISSN: 1364-5072.
AU Donaghy J; McKay A M
AN 1998111462 MEDLINE

L61 ANSWER 9 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 4
TI Release of covalently bound ferulic acid from fiber in the human colon
SO JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, (MAR 1997) Vol. 45, No. 3, pp. 661-667.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.
ISSN: 0021-8561.
AU Kroon P A (Reprint); Faulds C B; Ryden P; Robertson J A; Williamson G
AN 97:246009 SCISEARCH

L61 ANSWER 10 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI An Aspergillus awamori acetylesterase: purification of the enzyme, and cloning and sequencing of the gene
SO BIOCHEMICAL JOURNAL, (1 SEP 1997) Vol. 326, Part 2, pp. 485-490.
Publisher: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON, ENGLAND W1N 3AJ.
ISSN: 0264-6021.
AU Koseki T (Reprint); Furuse S; Iwano K; Sakai H; Matsuzawa H
AN 97:672342 SCISEARCH

L61 ANSWER 11 OF 135 HCAPLUS COPYRIGHT 2003 ACS
TI Cooperative effect of .beta.-xylanase and .alpha.-glucuronidase on Eucalyptus grandis kraft pulp bleaching
SO Brazilian Symposium on the Chemistry of Lignins and Other Wood Components, Proceedings, 5th, Curitiba, Aug. 31-Sept. 5, 1997 (1997), Volume 6, 430-437. Editor(s): Ramos, Luiz Pereira. Publisher: Universidade Federal do Parana, Departamento de Quimica, Curitiba, Brazil.
CODEN: 65HKA6
AU Duran, Nelson; De Toledo, Andrea R.; Milagres, Adriane M. F.; Esposito, Elisa; Curotto, Emilia; Angelo, Raquel
AN 1997:763924 HCAPLUS
DN 127:360125

L61 ANSWER 12 OF 135 HCAPLUS COPYRIGHT 2003 ACS
TI Differences in enzymic activities of cecal contents of rats fed on differently processed dietary fibers
SO Food Science and Technology International, Tokyo (1997), 3(4), 379-383
CODEN: FTINF2; ISSN: 1341-7592
AU Nishizawa, Chieko; Ohta, Takeo; Egashira, Yukari; Sanada, Hiroo
AN 1998:101625 HCAPLUS
DN 128:192011

L61 ANSWER 13 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 5
TI Novel biotransformations of agro-industrial cereal waste by **ferulic** acid **esterases**
SO INDUSTRIAL CROPS AND PRODUCTS, (AUG 1997) Vol. 6, No. 3-4, pp. 367-374.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.

ISSN: 0926-6690.
AU Faulds C B (Reprint); Bartolome B; Williamson G
AN 97:629485 SCISEARCH

L61 ANSWER 14 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Process for the isolation of preparative quantities of [2-O-(trans-feruloyl)-alpha-L-arabinofuranosyl]-(1->5)-L-arabinofuranose from sugarbeet
SO CARBOHYDRATE RESEARCH, (19 MAY 1997) Vol. 300, No. 4, pp. 351-354.
Publisher: ELSEVIER SCI LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, OXON, ENGLAND OX5 1GB.
ISSN: 0008-6215.
AU Kroon P A (Reprint); Conesa M T G; Colquhoun I J; Williamson G
AN 97:447363 SCISEARCH

L61 ANSWER 15 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 6
TI Hydrolysis and fermentation by rat gut microorganisms of 2-O-beta-D-xylopyranosyl-(5-O-feruloyl)-L-arabinose derived from grass cell wall arabinoxylan
SO JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE, (MAR 1997) Vol. 73, No. 3, pp. 296-300.
Publisher: JOHN WILEY & SONS LTD, BAFFINS LANE CHICHESTER, W SUSSEX, ENGLAND PO19 1UD.
ISSN: 0022-5142.
AU Wende G; Buchanan C J; Fry S C (Reprint)
AN 97:257808 SCISEARCH

L61 ANSWER 16 OF 135 HCAPLUS COPYRIGHT 2003 ACS
TI Enzymic release of ferulic acid from barley spent grain
SO Journal of Cereal Science (1997), 25(3), 285-288
CODEN: JCSCDA; ISSN: 0733-5210
AU Bartolome, B.; Faulds, C. B.; Williamson, G.
AN 1997:363722 HCAPLUS
DN 127:80516

L61 ANSWER 17 OF 135 MEDLINE DUPLICATE 7
TI Methyl phenylalkanoates as substrates to probe the active sites of esterases.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1997 Aug 15) 248 (1) 245-51.
Journal code: 0107600. ISSN: 0014-2956.
AU Kroon P A; Faulds C B; Brezillon C; Williamson G
AN 97454310 MEDLINE

L61 ANSWER 18 OF 135 MEDLINE DUPLICATE 8
TI Influence of ferulic acid on the production of **feruloyl esterases** by Aspergillus niger.
SO FEMS MICROBIOLOGY LETTERS, (1997 Dec 15) 157 (2) 239-44.
Journal code: 7705721. ISSN: 0378-1097.
AU Faulds C B; deVries R P; Kroon P A; Visser J; Williamson G
AN 1998096829 MEDLINE

L61 ANSWER 19 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 9
TI Purification of ferulic acid by adsorption after enzymic release from a sugar-beet pulp extract
SO INDUSTRIAL CROPS AND PRODUCTS, (AUG 1997) Vol. 6, No. 3-4, pp. 237-252.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0926-6690.
AU Couteau D; Mathaly P (Reprint)
AN 97:629471 SCISEARCH

L61 ANSWER 20 OF 135 MEDLINE DUPLICATE 10
TI An Aspergillus niger **esterase** (**ferulic** acid **esterase** III) and a recombinant Pseudomonas fluorescens subsp.

cellulosa esterase (Xy1D) release a 5-5' ferulic dehydrodimer (diferulic acid) from barley and wheat cell walls.
SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1997 Jan) 63 (1) 208-12. Journal code: 7605801. ISSN: 0099-2240.
AU Bartolome B; Faulds C B; Kroon P A; Waldron K; Gilbert H J; Hazlewood G; Williamson G
AN 97133958 MEDLINE

L61 ANSWER 21 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 11
TI Chemistry and biochemistry of hemicelluloses: Relationship between hemicellulose structure and enzymes required for hydrolysis
SO MACROMOLECULAR SYMPOSIA, (JUL 1997) Vol. 120, pp. 183-196. Publisher: HUTHIG & WEPF VERLAG, AUF DEM WOLF 4, CH-4052 BASEL, SWITZERLAND. ISSN: 1022-1360.
AU Puls J (Reprint)
AN 97:645075 SCISEARCH

L61 ANSWER 22 OF 135 MEDLINE DUPLICATE 12
TI Chemical and thermal stability of **ferulic** acid **esterase** -III from Aspergillus niger.
SO INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES, (1997 Aug) 21 (1-2) 163-7. Journal code: 7909578. ISSN: 0141-8130.
AU Williamson G; Vallejo J
AN 97426327 MEDLINE

L61 ANSWER 23 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Structure and functions of feruloylated polysaccharides
SO PLANT SCIENCE, (12 SEP 1997) Vol. 127, No. 2, pp. 111-127. Publisher: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND. ISSN: 0168-9452.
AU Ishii T (Reprint)
AN 97:624949 SCISEARCH

L61 ANSWER 24 OF 135 HCAPLUS COPYRIGHT 2003 ACS
TI Enzymes from barley which solubilize .beta.-glucan
SO Proceedings of the Congress - European Brewery Convention (1997), 26th, 75-82 CODEN: EBCPA6; ISSN: 0367-018X
AU Bamforth, C. W.; Moore, J.; McKillop, D.; Williamson, G.; Kroon, P. A.
AN 1998:548818 HCAPLUS
DN 129:272156

L61 ANSWER 25 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI PARTIAL-PURIFICATION AND CHARACTERIZATION OF A POLYPHENOL ESTERASE FROM ASPERGILLUS-NIGER
SO PROCESS BIOCHEMISTRY, (JAN 1997) Vol. 32, No. 1, pp. 61-69. ISSN: 0032-9592.
AU MADANI W; KERMASHA S (Reprint); GOETGHEBEUR M; TSE M
AN 96:817266 SCISEARCH

L61 ANSWER 26 OF 135 MEDLINE
TI Xylanolytic enzymes from fungi and bacteria.
SO CRITICAL REVIEWS IN BIOTECHNOLOGY, (1997) 17 (1) 39-67. Ref: 187 Journal code: 8505177. ISSN: 0738-8551.
AU Sunna A; Antranikian G
AN 97222601 MEDLINE

L61 ANSWER 27 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 13
TI Xylanase and **ferulic** acid **esterase** production by a wild strain of Aspergillus terreus
SO FOOD TECHNOLOGY AND BIOTECHNOLOGY, (JAN-MAR 1997) Vol. 35, No. 1, pp.

13-22.
Publisher: FACULTY FOOD TECHNOLOGY BIOTECHNOLOGY, UNIV ZAGREB, KACIECEVA 23, 41000 ZAGREB, CROATIA.
ISSN: 1330-9862.
AU Balogun S B (Reprint); Gomes J; Steiner W
AN 97:530732 SCISEARCH

L61 ANSWER 28 OF 135 HCAPLUS COPYRIGHT 2003 ACS
TI Differential host use of quaking aspen (Populus tremuloides) by three Lepidoptera (Papilio canadensis, Papilio glaucus, and Lymantria dispar): role of biochemical adaptations in **phenolic** glycoside tolerance (**esterase** inhibitors, glucosidases, antioxidants, gypsy moth, swallowtail butterfly)
SO (1996) 172 pp. Avail.: Univ. Microfilms Int., Order No. DA9708855
From: Diss. Abstr. Int., B 1997, 57(12), 7335
AU Thompson, Cynthia Platz
AN 1997:368479 HCAPLUS
DN 127:92952

L61 ANSWER 29 OF 135 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Enzyme system including **ferulic**-acid-**esterase** for treating plant material;
recombinant ferulate-esterase expression, optionally with e.g. peroxidase and endo-1,4-beta-D-xylanase, for ferulic acid production, polysaccharide treatment, etc.
AU Michelson B; de Vries R P; Visser J; Soe J B; Poulsen C H; Zargahi M R
AN 1997-01068 BIOTECHDS
PI GB 2301103 27 Nov 1996

L61 ANSWER 30 OF 135 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Acetyl esterases of Aspergillus niger: Purification and mode of action on pectins.
SO Visser, J. [Editor]; Voragen, A. G. J. [Editor]. Progress in Biotechnology, (1996) Vol. 14, pp. 793-798. Progress in Biotechnology; Pectins and pectinases.
Publisher: Elsevier Science Publishers B.V. PO Box 211, Sara Burgerhartstraat 25, 1000 AE Amsterdam, Netherlands.
Meeting Info.: International Symposium Wageningen, Netherlands December 3-7, 1995
ISSN: 0921-0423. ISBN: 0-444-82330-1.
AU Searle-Van Leeuwen, M. J. F. (1); Vincken, J.-P. (1); Schipper, D.; Voragen, A. G. J. (1); Beldman, G. (1)
AN 1997:187730 BIOSIS

L61 ANSWER 31 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI DEGRADATION OF PERENNIAL RYEGRASS LEAF AND STEM-CELL WALLS BY THE ANAEROBIC FUNGUS NEOCALLIMASTIX SP STRAIN CS3B
SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (APR 1996) Vol. 62, No. 4, pp. 1437-1440.
ISSN: 0099-2240.
AU SIJTSMA L (Reprint); TAN B
AN 96:292949 SCISEARCH

L61 ANSWER 32 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 14
TI **Ferulic** acid **esterase** catalyses the solubilization of beta-glucans and pentosans from the starchy endosperm cell walls of barley
SO BIOTECHNOLOGY LETTERS, (DEC 1996) Vol. 18, No. 12, pp. 1423-1426.
Publisher: CHAPMAN HALL LTD, 2-6 BOUNDARY ROW, LONDON, ENGLAND SE1 8HN.
ISSN: 0141-5492.
AU Moore J; Bamforth C W (Reprint); Kroon P A; Bartolome B; Williamson G
AN 97:22005 SCISEARCH

L61 ANSWER 33 OF 135 HCAPLUS COPYRIGHT 2003 ACS
TI Acetyl esterases of Aspergillus niger: purification and mode of action on

pectins
SO Progress in Biotechnology (1996), 14(Pectins and Pectinases), 793-798
CODEN: PBITE3; ISSN: 0921-0423
AU Searle-Van Leeuwen, M. J. F.; Vincken, J.-P.; Schipper, D.; Voragen, A. G. J.; Beldman, G.
AN 1997:87909 HCAPLUS
DN 126:154285

L61 ANSWER 34 OF 135 HCAPLUS COPYRIGHT 2003 ACS
TI Enzymic release of ferulic acid from sugar beet pulp using a specific esterase from Aspergillus niger
SO Progress in Biotechnology (1996), 14(Pectins and Pectinases), 761-768
CODEN: PBITE3; ISSN: 0921-0423
AU Kroon, P.A.; Faulds, C.B.; Brezillon, C.; Williamson, G.
AN 1997:87904 HCAPLUS
DN 126:154345

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L61 ANSWER 112 OF 135 MEDLINE DUPLICATE 56
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L61 ANSWER 117 OF 135 HCAPLUS COPYRIGHT 2003 ACS
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L61 ANSWER 118 OF 135 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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L61 ANSWER 134 OF 135 HCAPLUS COPYRIGHT 2003 ACS
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ANSWER SET L61 HAS BEEN SAVED AS 'FAE/A'

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,89,91,94,97,100,106,110,119,133

L61 ANSWER 4 OF 135 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB An enzyme was purified from a commercial Aspergillus niger enzyme preparation which was similar to FAE-III, a **ferulic**-acid-**esterase** purified from A. niger with high activity against methyl esters of several cinnamic acids. The corresponding gene (faeA) from A. niger and Aspergillus tubingensis was cloned using reverse genetics and both gene products were overexpressed. The A. niger faeA product had identical characteristics to FAE-III, ie. they were the same enzyme, as determined by mol.wt., pH and temp. optima, pI, N-terminal sequence and activity on methyl formate. Overexpression of faeA protein showed that the A. tubingensis enzyme was more sensitive to degradation than the A. niger enzyme, despite a 93% similarity in sequence. A. tubingenesis faeA protein may be more sensitive to acid proteases. faeA protein could release ferulic acid from wheat arabinoxylan and from sugar beet pectin without the aid of other enzymes; the faeA gene was induced by growth on these substrates. Addition of A. niger endo-1,4-beta-D-xylanase (EC-3.2.1.8) increased the amount of xylanase released from arabinoxylan 2 to 7-fold. (42 ref)

L61 ANSWER 5 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI
AB Acetylesterase and cinnamoyl ester hydrolase activities were demonstrated in culture supernatant of the anaerobic ruminal fungus Neocallimastix patriciarum. A cDNA expression library from N. patriciarum was screened for esterases using beta-naphthyl acetate and a model cinnamoyl ester compound. cDNA clones representing four different esterase

genes (bnaA-D) were isolated. None of the enzymes had cinnamoyl ester hydrolase activity, but two of the enzymes (BnaA and BnaC) had acetylxylan esterase activity. bnaA, bnaB and bnaC encode proteins with several distinct domains. Carboxy-terminal repeats in BnaA and BnaC are homologous to protein-docking domains in other enzymes from Neocallimastix species and another anaerobic fungus, a Piromyces sp. The catalytic domains of BnaB and BnaC are members of a recently described family of Ser/His active site hydrolases [Upton, C. & Buckley, J.T. (1995). Trends Biochem Sci 20, 178-179]. BnaB exhibits 40% amino acid identity to a domain of unknown function in the CelE cellulase from Clostridium thermocellum and BnaC exhibits 52% amino acid identity to a domain of unknown function in the XynB xylanase from Ruminococcus flavefaciens. BnaA, whilst exhibiting less than 10% overall amino acid identity to BnaB or BnaC, or to any other known protein, appears to be a member of the same family of hydrolases, having the three universally conserved amino acid sequence motifs. Several other previously described esterases are also shown to be members of this family, including a rhamnogalacturonan acetylesterase from Aspergillus aculeatus. However, non of the other previously described enzymes with acetylxylan esterase activity are members of this family of hydrolases.

L61 ANSWER 10 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI
AB An inducible acetylesterase was purified from the culture medium of Aspergillus awamori strain IFO4033 growing on wheat-bran culture by ion-exchange, gel-filtration and hydrophobic-interaction chromatographies. The purified enzyme had an M-r of 31000 and contained Asn-linked oligosaccharides. The enzyme liberated acetic acid from wheat bran, hydrolysed only alpha-naphthyl acetate and propionate when aromatic esters were used for the substrate, and was tentatively classified as a carboxylic esterase (EC 3.1.1.1). The gene encoding acetylesterase was cloned and sequenced. The deduced amino acid sequence showed that acetylesterase was produced as a 304-amino-acid-residue precursor, which was converted post-translationally into a 275-amino-acid-residue mature protein. Part of the sequence of acetylesterase was similar to the region near the active-site serine of lipases of Geotrichum candidum and Candida cylindracea. A unique site of putative Asn-linked oligosaccharides was presented.

L61 ANSWER 13 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 5
AB With the yearly accumulation of agro-industrial waste-material generated by the milling, brewing and sugar industries in Europe, the importance of extracting high value residues must be considered to offset the cost of treating and disposing of the residues. This work reviews the identification, purification and characterisation of novel microbial esterases capable of releasing the bioactive phenolic compound, ferulic acid, from cereal cell-walls and agro-industrial waste. These phenolic residues restrict the extent of hydrolysis of cell-wall carbohydrates. Potential applications for the esterases in the food and pharmaceutical industries are described. Enzymic removal of ferulic acid is very mild, allowing further treatment/processing of the residue and removes the need for environmentally-unfriendly chemical clean-up processes. We now report the hydrolysis, on a laboratory scale, of wheat bran (1 kg) by a Trichoderma xylanase preparation and an Aspergillus niger **ferulic** acid **esterase** (FAE-III) to produce free ferulic acid (5.7 g).

L61 ANSWER 14 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI
AB Specific enzymes were used to hydrolyse sugarbeet pulp to facilitate the isolation of [2-O-(trans-feruloyl)-alpha-L-arabinofuranosyl]-(1 --> 5)-L-arabinofuranose in preparative amounts. The feruloylated arabinose disaccharide was purified by chromatography on Sephadex LH-20 and Bio-Gel P-2 and the structure confirmed by NMR and UV spectroscopy and high-performance liquid chromatography.

L61 ANSWER 16 OF 135 HCAPLUS COPYRIGHT 2003 ACS

AB **Ferulic** acid **esterase** and xylanase act together to release ferulic acid from spent grain. The extent of release is lower than wheat bran but higher than maize bran, presumably due to the branching of the resp. arabinoxylans.

L61 ANSWER 19 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 9
AB An aqueous sugar-beet pulp extract obtained by pressure-cooking and mainly composed of pectic fragments, was treated with a pectinolytic preparation. A strong, but limited depolymerization occurred fastly, while **feruloyl-esterase** activities appeared more gradually, pointing to some deficiencies of the enzymic mix. The solubility of ferulic acid in the sugar-rich, acidic hydrolysate proved stable, and no crystallization could be triggered in this complex medium. Comparative batch tests were carried out with preselected commercially-available adsorbents: activated carbons, polystyrenic divinylbenzene-crosslinked resins, and polyvinylpolypyrrolidone (PVPP). In each case, the ferulic acid uptake is lower at pH values above 4.5, the pKa determined for its carboxylic group. The natural acidity of the hydrolysate, pH 3.4, was chosen as the optimum. The chemically-activated carbon (SA1817) has the highest affinity for ferulic acid, while Amberlite XAD-16 performs best among the polystyrenic resins. PVPP also offers an appreciable adsorption efficiency. Selectivity is ensured, since only the charcoal removes a slight amount of galacturonic acid, which is released on rising the pH to 4. Alkaline dissociation and ethanolic extraction were investigated as desorption strategies, and ethanol was kept as the most suitable common eluent. Dynamic column studies allowed to estimate the maximum specific capacity at about 22, 12 and 8% (w:w ferulic acid/adsorbent) for the chemical granular activated carbon (GAG), XAD-16 and PVPP, respectively. Ferulic acid is quantitatively recovered after ethanolic elution of the three selected adsorbents, and the best purity is achieved with PVPP, followed by GAG. (C) 1997 Elsevier Science B.V.

L61 ANSWER 20 OF 135 MEDLINE DUPLICATE 10
AB Diferulate esters strengthen and cross-link primary plant cell walls and help to defend the plant from invading microbes. Phenolics also limit the degradation of plant cell walls by saprophytic microbes and by anaerobic microorganisms in the rumen. We show that incubation of wheat and barley cell walls with **ferulic** acid **esterase** from Aspergillus niger (FAE-III) or Pseudomonas fluorescens (XylD), together with either xylanase I from Aspergillus niger, Trichoderma viride xylanase, or xylanase from Pseudomonas fluorescens (XylA), leads to release of the ferulate dimer 5-5' diFA [(E,E)-4,4'-dihydroxy-5,5'-dimethoxy-3,3'-bicinnamic acid]. Direct saponification of the cell walls without enzyme treatment released the following five identifiable ferulate dimers (in order of abundance): (Z)-beta-(4-[(E)-2-carboxyvinyl]-2-methoxyphenoxy)-4-hydroxy-3-methoxycinnamic acid, trans-5-[(E)-2-carboxyvinyl]-2-(4-hydroxy-3-methoxy-phenyl) -7-methoxy-2, 3-dihydrobenzofuran-3-carboxylic acid, 5-5' diFA, (E,E)-4, 4'-dihydroxy-3, 5'-dimethoxy-beta, 3'-bicinnamic acid, and trans-7-hydroxy-1-(4-hydroxy-3-methoxyphenyl) -6-methoxy-1, 2-dihydronaphthalene-2, 3-dicarboxylic acid. Incubation of the wheat or barley cell walls with xylanase, followed by saponification of the solubilized fraction, yielded 5-5'diFA and, in some cases, certain of the above dimers, depending on the xylanase used. These experiments demonstrate that FAE-III and XYLD specifically release only esters of 5-5'diFA from either xylanase-treated or insoluble fractions of cell walls, even though other esterified dimers were solubilized by preincubation with xylanase. It is also concluded that the esterified dimer content of the xylanase-solubilized fraction depends on the source of the xylanase.

L61 ANSWER 22 OF 135 MEDLINE DUPLICATE 12
AB The stability of **ferulic** acid **esterase** III (FAE-III) from Aspergillus niger was examined using chemical and thermal denaturation. Thermal denaturation was irreversible and the loss of

activity was dependent on pH. At 60 degrees C and pH 6.0, the rate constant of unfolding was 0.76 10(-3)/s, and the change in free energy of irreversible inactivation, deltaG*, was 101.9 kJ/mol. Sinapic acid, a product of the reaction of methyl sinapate with FAE-III, reduced the rate of unfolding (0.66 10(-3)/s at 0.1 mM sinapic acid). Chemical denaturation was performed using guanidine hydrochloride. FAE-III was very sensitive to this denaturant, and the midpoint of unfolding was 1.38 M guanidine hydrochloride at 30 degrees C, pH 6.0. The stability of FAE-III is compared to other enzymes.

L61 ANSWER 25 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI

AB Crude pectinase extract (FI), obtained from Aspergillus niger, was partially purified by ammonium sulphate precipitation at saturation of 0-20% (FIIa), 20-80% (FIIb) and 80-100% (FIIc). While all precipitated fractions exhibited pectin methyl esterase (PME), beta-1,3-glucanase, polyphenol esterase (PPE), polygalacturonase (PG) and beta-galactosidase activities, fraction FIIa contained the majority of PME and beta-1,3-glucanase activities. However, fraction FIIc contained the highest PPE, PG and beta-galactosidase activities, whereas fraction FIIb contained the least of all these activities. Electrophoretic analyses of the partially purified FIIc fraction demonstrated the presence of one major band with a molecular weight of 17.5 kDa and five minor bands with molecular weights in the range from 7.2 to 20.1 kDa. The purification procedure resulted in a 2.1-fold increase in PPE activity compared to that of the crude extract. The optimum pH and temperature for PPE hydrolytic activity were 5.25 and 40 degrees C, respectively. PPE reduced the oxidation, by mushroom tyrosinase, of catechin and chlorogenic acid used as substrates to brownish coloured products, however, the rate of oxygen uptake during the oxidation reaction was constant. The PPE inhibitory effect on tyrosinase activity was 12 times higher with catechin than with chlorogenic acid. In addition, PPE exhibited a competitive and a mixed type of inhibition for tyrosinase with chlorogenic acid and catechin, respectively High-performance liquid chromatography analyses of enzymic end-products suggested that, in the case of chlorogenic acid, the inhibitory mechanism of PPE was probably due to the formation of substances that acted as inhibitors to tyrosinase activity. Moreover, the results indicated that the inhibitory mechanism of PPE on tyrosinase, using catechin as substrate, was not the same as that for chlorogenic acid. Copyright (C) 1996 Elsevier Science Ltd

L61 ANSWER 27 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 13

AB The medium composition for the production of endo-xylanase and **ferulic** acid **esterase** in shake-flask cultures by Aspergillus terreus was optimized using statistical methods. The optimized medium composition for xylanase production was found to be 40 g L-1 coarse corn cobs (c. 1-5 mm), 4.5 g L-1 gelitaflex, 12 g L-1 NnNO(3), while that for **ferulic** acid **esterase** production was 12 g L-1 oat spelt xylan, 6.4 g L-1 soybean meal and 4 g L-1 NH4NO3. The optimized media and culture conditions gave a maximum of 538.3 U/mL i.e. 8973 nkat/ml xylanase and 0.7 U/mL i.e. 11.7 nkat/mL, **ferulic** acid **esterase** activities after 5-7 days shake cultures. In addition to xylanase and **ferulic** acid **esterase** activities, the culture filtrates exhibited low or appreciable levels of filter-paper cellulase, carboxymethyl cellulase, beta-glucosidase, beta-xylosidase and acetyl esterase activities. In a laboratory bioreactor (10 L) culture using fine corn cobs (0.25-0.50 mm), xylanase production was slightly better than in the parallel shake-flask cultures. The pH optima of xylanase, beta-xylosidase and **ferulic** acid **esterase** were 5.0, 4.5 and 5.0-6.0, respectively. The optimum temperature for xylanase, beta-xylosidase and **ferulic** acid **esterase** was 50 degrees C. Xylanase anti beta-xylosidase showed moderate pH and thermal stabilities.

L61 ANSWER 30 OF 135 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

L61 ANSWER 32 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 14
AB Two separate, highly purified **ferulic** acid **esterases** from a fungal and bacterial source are both capable of releasing beta-glucan and pentosans from the cell wails of the starchy endosperm of barley. This suggests that ester linkages involving ferulic acid contribute to the integrity of such walls.

L61 ANSWER 34 OF 135 HCAPLUS COPYRIGHT 2003 ACS
AB We have purified and characterized a novel esterase (CinnAE) from Aspergillus niger. The enzyme demonstrated activity towards various sol. feruloylated oligosaccharides derived from sugar beet pulp (SBP) but, when acting alone, the esterase released only 0.9% of the alkali-extractable ferulic acid from SBP. However, when incubated with a mixt. of endo-arabinanase and .alpha.-L-arabinofuranosidase, there was a 14-fold increase in ferulic acid release, demonstrating a strong synergy between these three enzymes. No increase in ferulic acid release was obsd. when SBP was incubated with CinnAE plus endo-(1,4)-.beta.-D-galactanase and .beta.-D-galactosidase. Hence, feruloylated arabinans in SBP are readily available for hydrolysis by arabinan-degrading enzymes, whereas feruloylated galactans are not available for hydrolysis by galactan-degrading enzymes.

L61 ANSWER 37 OF 135 MEDLINE DUPLICATE 15

L61 ANSWER 39 OF 135 MEDLINE DUPLICATE 16

L61 ANSWER 41 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 17
AB A **ferulic** acid **esterase** (FAE-III), which was induced by growth of Aspergillus niger CBS 120.49 on oat-spelts xylan, was capable of releasing ferulic acid from wheat bran but not from sugar-beet pulp (SBP) [Faulds CB, Williamson G (1994) Microbiology 140:779-787]. Growth of this strain on SBP gave low levels of **ferulic** acid **esterase** activity (using methyl ferulate as substrate). A similar growth with a different A. niger strain (CS 180) gave tenfold higher levels of esterase activity. Assaying culture filtrates obtained from A. niger CS 180 grown on SBP over a 3 to 10-day period against four simple phenolic methyl esters demonstrated that at least two esterases were produced, and, by comparison of substrate specificity. FAE-III was either absent or present only at low levels. Furthermore, immunodetection of proteins did not detect the presence of FAE-III in culture supernatants of SBP-grown cultures, whereas it did ill cultures grown on oat-spelts xylan. These results show that SBP does not contain the inducer for FAE-III, but does induce novel esterases. When A. niger CS 180 cultures were grown on different carbon sources, esterase activity was induced on SBP, sugar-beet arabinan and oat-spelts xylan, but not on simple sugars or de-esterified sugarbeet pectin. Further, SBP-grown cultures co-inoculated with arabinanase, galactanase or xylanase did not exhibit increased levels of extracellular FAE activity or an earlier appearance of esterase activity, although there was an increase in esterase activity with added polygalacturonase. These results show that novel esterases are induced by growth of A. niger on SBP.

L61 ANSWER 42 OF 135 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB A **ferulic** acid **esterase** (FAE-III), which was induced by Aspergillus niger CBS 120.49 growth on oat-spelts xylan (OSX), was capable of releasing ferulic acid from wheat bran but not from sugarbeet pulp (SBP). Growth on SBP gave low levels of **ferulic** acid **esterase** activity using methylferulate as a substrate. Similar growth was observed for A. niger CS 180, resulting in 10-fold higher **ferulic** acid **esterase** levels. SBP-grown CS 180 3- to 10-day-old culture filtrates were assayed against 4 phenolic methyl esters, demonstrating that at least 2 esterases were produced. The substrate specificity of FAE-III was either absent or present at low

levels. Protein immunodetection did not detect FAE-III in SBP-grown culture supernatants, whereas it did in OSX-grown cultures. Results showed that SBP did not contain the inducer for FAE-II but induced novel esterases. When CS 180 was grown on different C-sources, esterase activity was induced on SBP, sugarbeet arabinan and OSX but not on simple sugars or deesterified sugarbeet pectin. Thus, novel esterases are induced by A. niger growth on SBP. (28 ref)

L61 ANSWER 43 OF 135 MEDLINE DUPLICATE 18
AB Aspergillus niger **cinnamoyl esterase** (CinnAE) is shown to be active towards a wide range of feruloylated oligosaccharides derived from sugar-beet pulp (SBP). The **esterase** hydrolysed **ferulic** acid ester-linked to either C-2 of arabinose or C-6 of galactose residues, and demonstrated the highest activity towards the feruloylated arabinose trisaccharide. However, CinnAE was able to release only 0.88% of total alkali-extractable ferulic acid from SBP in 24 h when acting alone. To determine whether cell-wall-degrading enzymes could increase the release of ferulic acid by CinnAE, SBP was incubated with various carbohydrases [cellulase, polygalacturonase, endo-arabinanase, alpha-L-arabinofuranosidase, endo-(1,4-beta-D-galactanase, beta-D-galactosidase]. These were added alone and in pairs, both in the presence and absence of CinnAE. We showed that all the carbohydrases tested were free of esterase activity. When individual carbohydrases were incubated with SBP, whether in the presence or absence of CinnAE, less than 1% of the feruloyl groups were released. When incubated with a mixture of endo-arabinanase and alpha-L-arabinofuranosidase, the esterase was able to release 14 times more of the alkali-extractable ferulic acid present in the whole pulp as free acid than CinnAE alone. Ferulic acid is linked either to L-arabinose or D-galactose in SBP, but no corresponding increase in ferulic acid release was detected when SBP was incubated with CinnAE plus endo-(1,4)-beta-D-galactanase and beta-D-galactosidase (both from A. niger). Hence feruloylated arabinans in SBP are readily available for hydrolysis by arabinan-degrading enzymes, whereas feruloylated galactans are not available for hydrolysis by galactan-degrading enzymes.

L61 ANSWER 44 OF 135 MEDLINE DUPLICATE 19
AB An inducible esterase has been isolated from a liquid culture of Aspergillus niger grown on sugar-beet pulp. The enzyme was active on methyl esters of cinnamic acids, caffeic > p-coumaric > ferulic, and is therefore termed a **cinnamoyl esterase**. The enzyme was not active on methyl sinapinate, a good substrate for **ferulic** acid **esterase** III, which was purified previously from A. niger [Faulds and Williamson (1994) Microbiology 140, 779-787]. With methyl caffeate as substrate the enzyme had temperature and pH optima of 50 degrees C and 6.0 respectively, and a specific activity of 96.9 units per mg of protein. The purified protein (native molecular mass 145 000 Da) gave a single heavily stained band on SDS/PAGE, suggesting the protein was a dimer, and seemed to be heavily glycosylated. Isoelectric focusing gave a single band corresponding to a pI of 4.80. The pure enzyme was free of other carbohydrase activities. The activity of the pure enzyme was inhibited by more than 99% after treatment with the serine-specific protease inhibitor aminoethylbenzenesulphonylfluoride (1 mM) for 12 h. The enzyme was capable of releasing ferulic acid from sugar beet pulp.

L61 ANSWER 45 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 20
AB A rapid, simple and sensitive method for detection of ferulic and p-coumaric acids using HPLC has been developed which can be used to determine the respective **phenolic** acid **esterase** activities of microorganisms. Prior concentration, purification or derivatization of the samples are not required. As little as 0.5 mg ferulic or p-coumaric acid/l could be detected and estimated in <1 h. The method is specific for the two phenolic acids since no interference by other components was observed.

L61 ANSWER 50 OF 135 HCAPLUS COPYRIGHT 2003 ACS
AB A review with 18 refs.

L61 ANSWER 52 OF 135 MEDLINE DUPLICATE 22
AB Ferulic acid was efficiently released from a wheat bran preparation by a **ferulic** acid **esterase** from Aspergillus niger (FAE-III) when incubated together with a Trichoderma viride xylanase (a maximum of 95% total ferulic acid released after 5 h incubation). FAE-III by itself could release ferulic acid but at a level almost 24-fold lower than that obtained in the presence of the xylanase (2 U). Release of ferulic acid was proportional to the FAE-III concentration between 0.1 U and 1.3 U, but the presence of low levels of xylanase (0.1 U) increased the amount of ferulic acid released 6-fold. Total sugar release was not influenced by the action of FAE-III on the wheat bran, but the rate of release of the apparent end-products of xylanase action (xylose and xylobiose) was elevated by the presence of the esterase. The results show that FAE-III and the xylanase act together to break down feruloylated plant cell-wall polysaccharides to give a high yield of ferulic acid.

L61 ANSWER 53 OF 135 MEDLINE DUPLICATE 23
AB Extracellular esterase production by Penicillium expansum, Penicillium brevicompactum and Aspergillus niger was determined in both liquid and solid-state culture. Methyl ferulate was used as the main carbon source in liquid culture whereas wheat bran and sugar beet pulp were used in solid-state culture. Extracted enzyme for each fungus showed activity in the presence of ONP butyrate, methyl ferulate, methyl coumarate and two 'natural' feruloylated carbohydrate esters. Higher enzyme recoveries were obtained using wheat bran in solid-state culture. Higher levels of **feruloyl esterase** activity were recovered from P. expansum on all feruloylated substrates than from P. brevicompactum or A. niger. Using ONP butyrate as substrate the pH and temperature optima for the esterases of both Penicillium spp. were 6.0 and 25-30 degrees C. Aspergillus niger esterase activity showed a broader temperature range with an optimum at 40 degrees C.

L61 ANSWER 55 OF 135 MEDLINE DUPLICATE 24

L61 ANSWER 56 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 25
AB We have examined two A. niger **esterases** (FAE-III (**ferulic** acid **esterase** III) and CinnAE (**cinnamic** acid **esterase**)) for their ability to release ferulic acid from maize bran, a particularly rich source of ferulic acid. However, even though both enzymes exhibited significant activites on novel feruloylated oligosaccharides derived from maize bran (including FAXX (O-(5-O-feruloyl-alpha-L-arabinofuranosyl)-(1-->3) -O-beta-xylopyranosyl-(1-->4)-D-xylopyranose)), neither esterase was efficient in removing ferulic acid from the whole bran, even in the presence of other carbohydrases. It is shown that the kinetics of ferulic acid release from feruloylated oligosaccharides was influenced by (a) the nature of the sugar-sugar linkage, and (b) the type of sugar present. The results suggest that enzymic release of ferulic acid from maize bran is limited by physical and steric factors, not by the chemical nature of the linkage.

L61 ANSWER 58 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 26
AB Ferulic and p-coumaric acid can be separated from their corresponding aliphatic methyl esters by capillary zone electrophoresis, which allows the convenient determination of **feruloyl** and p-**coumaroyl** **esterase** activities using synthetic esters as substrates. A feruloyl-containing sugar ester from wheat bran was also efficiently separated and used as substrate for the enzyme assays. Penicillium expansum was shown to produce **feruloyl**/p-**coumaroyl** **esterase** activity when grown on wheat bran in solid-state culture.

L61 ANSWER 62 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 28

AB **Feruloyl esterase** and p-**coumaroyl esterase** activities were separated by ion-exchange chromatography on a column of DEAE-Sepharose CL 6B and then purified by hydrophobic interaction chromatography and anion-exchange chromatography, respectively. **Feruloyl esterase** had an apparent molecular mass of 112 kDa (SDS-PAGE) and an isoelectric pH of 3.7; the p-**coumaroyl esterase** was much smaller (75 kDa) and had a pI of 4.2. The **feruloyl esterase** was highly specific for the methyl ester of ferulic acid and for feruloyl esters associated with a water-soluble xylan polysaccharide from wheat straw; p-**coumaroyl esterase** was also highly specific for the corresponding p-coumaroyl ester substrates. The wheat straw xylan was de-esterified by the esterases without prior degradation of the polysaccharide; however esterified short-chain xylooligosaccharides which were generated by a purified fungal xylanase were better substrates. The apparent K-m values for the feruloyl and p-coumaroyl residues were 0.93 +/- 0.05 and 0.031 +/- 0.02 mM, respectively using the methyl ester substrates; the respective V-max values wa e 2.82 +/- 0.05 and 0.57 +/- 0.03 mu mol min(-1) mg(-1) protein. The esterases had only a small capacity to release ferulic and p-coumaric acids from cell walls of various plants, including mesophyll cell walls from lye grass, but they were more effective when acting in concert with the commercially available plant cell wall-degrading enzyme Celluclast.

L61 ANSWER 63 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 29

AB An inducible **ferulic** acid **esterase** (FAE-III) has been isolated, purified and partially characterized from Aspergillus niger after growth on oat spelt xylan. The purification procedure utilized ammonium sulphate precipitation, hydrophobic interaction and anion-exchange chromatography. The purified enzyme appeared almost pure by SDS-PACE, with an apparent M(r) of 36000. A single band, corresponding to a pl of 3.3 was observed on isoelectric focusing. With methyl ferulate as substrate, the enzyme had a specific activity of 67 IU (mg protein)(-1), pH and temperature optima of 5 and 55-60 degrees C, respectively, and a K-m of 2.08 mM and a V-max of 175 mu mol min(-1) (mg protein)(-1). The enzyme was also active upon methyl sinapinate, methyl-3,4-dimethoxy cinnamate and methyl p-coumarate, but not benzoic acid methyl esters or methyl caffeate. Similarly, Streptomyces olivochromogenes FAE showed activity against methyl ferulate, methyl sinapinate and methyl p-coumarate, but at a level 420-fold less (on methyl ferulate) than the A. niger esterase. No activity was detected against the benzoate methyl esters. For both enzymes, this shows the necessity for C-3 on the phenol ring to be methoxylated and the aliphatic region of the substrate to be unsaturated. The specific activity of FAE-III on destarched wheat bran was 31 U (mg protein)(-1) in the presence of Trichoderma viride xylanase and 3 U (mg protein)(-1) in the absence. Apparent pH dependent binding of A. niger FAE-III to microcrystalline cellulose was also demonstrated.

L61 ANSWER 64 OF 135 MEDLINE DUPLICATE 30

AB The activity of two forms of **ferulic** acid **esterase** (FAE) from Aspergillus niger on a synthetic feruloylated substrate (methyl ferulate) and on 11 different feruloylated oligosaccharides from sugar-beet pulp and wheat bran was determined. The enzymes exhibited different specificities for the various feruloylated substrates and were more active on certain substrates of cell-wall origin than on methyl ferulate. Both enzymes preferred the arabinose residue to which ferulic acid is attached in the furanose form. FAE-I had no clear preference for the type of linkage involved between the ferulic acid units and the oligosaccharide chain. In contrast, FAE-III had a clear requirement for ferulic acid to be attached to O-5 of the Ara f ring while no catalysis was observed when ferulic acid was attached to O-2. Both enzymes showed maximum activity on feruloylated trisaccharides. An increase in the length of the oligosaccharide chain did not preclude catalysis, but feruloylated oligosaccharides of a dp > 3 were hydrolysed at a reduced

rate. Our results support the hypothesis that different kinds of **ferulic** acid **esterases** exist with different specificities for the oligosaccharide chain of the feruloylated substrates.

L61 ANSWER 72 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 34
AB High levels of 4-vinylphenol and 4-vinylguaiacol were detected in wines made from grape juice initially treated with some enzyme preparations. Two enzyme activities, which operate successively, are responsible for this formation. First, the **cinnamoyl esterase** activity from enzyme preparation liberates cinnamic acids from their corresponding tartaric acid esters. Second, cinnamic acids are transformed into 4-vinylphenol and 4-vinylguaiacol by decarboxylase activity provided by the yeasts. This activity is quite stable throughout alcoholic fermentation. The high levels of volatile phenols in some enzymatically treated wines could be responsible for unpleasant phenolic off-flavors. During storage, these compounds decreased and corresponding ethoxyethylphenols increased.

L61 ANSWER 74 OF 135 MEDLINE DUPLICATE 35
AB This review focuses on the description of recently discovered esterase enzymes involved in xylan degradation (acetyl xylan, **feruloyl**, and p-**coumaroyl esterases**). The occurrence of these enzymes in various microorganisms, assays used for determination of their activity, induction and production on different substrates, interaction with other xylanolytic enzymes, mode of action, substrate specificity, and biochemical characteristics are presented. The nature of substrates on which acetyl xylan **esterase**, **feruloyl**, and p-**coumaroyl esterase** are active and their role in xylan hydrolysis is emphasized. The potential applications of xylan-debranching esterases are outlined and their significance to applied microbiology is discussed.

L61 ANSWER 76 OF 135 MEDLINE DUPLICATE 37
AB Two forms of **ferulic** acid **esterase** from Aspergillus niger have been isolated from a commercial source of pectinase. One, designated I, has a M(r) of 132,000, is probably dimeric, and has a pI of 3.0. The second, designated II, was partially purified and is monomeric (M(r) 29,000), with a pI of 3.6. Both enzymes were free of pectinase and xylanase activity and released ferulic acid from methyl ferulate. In association with a xylanase, they also released ferulic acid from destarched wheat bran. **Ferulic** acid **esterase** II released a small amount of ferulic acid (0.09 unit/mg of protein) in the absence of xylanase. The enzymes had different specificities for a range of methyl ester derivatives of cinnamoyl and benzoyl acids, acetylated xylan and p-nitrophenyl acetate.

L61 ANSWER 89 OF 135 HCAPLUS COPYRIGHT 2003 ACS
AB Aspergillus niger produces a **ferulic** acid **esterase** (FAE) which interacts synergistically with Trichoderma viride xylanase to release ferulic acid from wheat bran. When incubated with a carbohydrase mixt. from Penicillium funiculosum, partial inhibition of all enzymic activity is seen. Using sugar beet pectin as a substrate, ferulic acid is released by FAE, but no synergism is obsd. with pectinase. The release of ferulic acid from plant cell wall polysaccharides by FAE is thus dependent upon (1) interaction with other carbohydrases, and (2) accessibility of the feruloyl group on the polymer side-chain.

L61 ANSWER 91 OF 135 MEDLINE DUPLICATE 44

L61 ANSWER 94 OF 135 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB Esterases are needed for their complete hydrolysis of xylans since these heteropolysaccharides are linked together not only by glycosidic bonds but also by ester linkages. As the esterified acetyl or phenolic

substituents are important for the functional properties of xylans, the actions of esterases may change the physical properties of the polymer. Knowledge of xylanolytic esterases has increased markedly in the past few years, and many microorganisms have been reported to produce multiple acetylesterases (EC-3.1.1.6) with different substrate specificities. Acetylesterases act synergistically with endo-1,4-beta-D-xylanases (EC-3.2.1.8) and with beta-xylosidase (EC-3.2.1.17) for the production of xylose from acetylated xylan. The presence of an esterase liberating phenyl side groups from cereal xylans was first detected in 1987, and the only enzyme purified hitherto had a broad substrate specificity, also liberating acetic acid from xylan. The acetyl-xylan-esterase and acetylesterase of Trichoderma reesei, the esterase of Aspergillus oryzae, and the **feruloyl-esterase** of Aspergillus niger were discussed. (49 ref)

L61 ANSWER 97 OF 135 MEDLINE DUPLICATE 46
AB A 4-hydroxy-3-methoxycinnamic acid (**ferulic** acid) **esterase** has been purified from the extracellular broth of cultures of Streptomyces olivochromogenes after growth on oat splet xylan. The purification procedure utilizes ion exchange on DEAE-BioGel A, anion exchange on Mono Q, gel filtration and hydrophobic interaction chromatography. The purified enzyme appeared as a single band on SDS-PAGE, with an apparent Mr of 29,000. Two bands, at pI7.9 and 8.5, were observed on isoelectric focusing. With methyl ferulate as substrate, the pH and temperature optima were 5.5 and 30 degrees C respectively, with a Km of 1.86 mM and Vmax of 0.3 mumols min-1 mg-1. The purfied enzyme released ferulic acid from de-starched wheat bran only in the presence of xylanase.

L61 ANSWER 100 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISI
AB Xylans are heteropolysaccharides which are depolymerized by beta-1,4-D-endoxylanases. Due to the abundance and variety of substituents in native xylans, different accessory enzymes are also needed for the total hydrolysis of xylan. The knowledge of alpha-glucuronidase, alpha-arabinosidase, and acetyl xylan- and **feruloyl esterases** has increased considerably in recent years. In addition to acting in synergism with endoxylanases and beta-xylosidase for the complete hydrolysis of xylan, some of these accessory enzymes are also capable of changing the structure of polymeric xylans.

L61 ANSWER 106 OF 135 LIFESCI COPYRIGHT 2003 CSA DUPLICATE 53
AB Production of extracellular **esterase** cleaving **phenolic** sidegroups from xylan was investigated using strains of Aspergillus . The activity levels produced were unrelated to the ferulic acid content of the lignocellulosic raw material in the cultivation medium. No correlation between the production of **feruloyl esterase**, acetyl xylan **esterase** and acetyl esterase activities was observed either. An esterase produced by Aspergillus oryzae was purified to electrophoretic homogeneity. The enzyme was an acidic monomeric protein having an isoelectric point of 3.6 and a molecular mass of 30 kDa. It was most active in the pH-range from 4.5 to 6.0 and was stable at temperatures up to 45 degree C. The esterase had a wide substrate specificity, liberating ferulic, p-coumaric and acetic acids from steam-extracted wheat straw fragments and acetic acid from acetylated xylo-oligomers and alpha -naphthyl acetate.

L61 ANSWER 110 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 55

L61 ANSWER 119 OF 135 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 60

L61 ANSWER 133 OF 135 HCAPLUS COPYRIGHT 2003 ACS
AB A. flavus produced an extracellular **esterase** that hydrolyzed **phenolic** carboxylic acid acyl esters. An assay based upon the measurement of the rate of phloroglucinol release on hydrolysis of the

ester of phloroglucinol and protocatechuic acid was described. The most active prepn. hydrolyzed 30.8 micromoles substrate/min/mg protein and was active against a wide range of esters of m- and p-hydroxybenzoic acid derivs. The enzyme was a homogeneous protein and had an is oelec. point of 4.45. The mol. wt. was 166,000. The enzyme was a glycoprotein contg. 42.8% carbohydrate; the amino acid compn. was described.

=>
=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	80.33	383.18

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-3.26	-3.26

STN INTERNATIONAL LOGOFF AT 16:25:38 ON 01 MAY 2003

PGPUB-DOCUMENT-NUMBER: 20030054510

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030054510 A1

TITLE: Process for the preparation of substituted 3-phenyl-propanoic acid esters and substituted 3-phenyl-propanoic acids

PUBLICATION-DATE: March 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Deussen, Heinz-Josef W.	Soborg		DK	
Zundel, Magali	Soborg		DK	

APPL-NO: 10/ 131876

DATE FILED: April 24, 2002

RELATED-US-APPL-DATA:

child 10131876 A1 20020424

parent continuation-of 09633890 20000807 US ABANDONED

non-provisional-of-provisional 60148504 19990812 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 1999 01100	1999DK-PA 1999 01100	August 5, 1999

US-CL-CURRENT: 435/135

ABSTRACT:

The present invention relates to a process comprising hydrolysis or trans esterification of one of the two enantiomeric forms of a racemic or enantiomerically enriched ester of formula I or IV by a higher rate than the other by an enzyme to give an ester and a acid (III) or two different esters (V) and (VI) with different R groups both with increased enantiomeric purity and a esterification process of a racemic or enantiomerically enriched acid (VII) by an enzyme to give an ester and an acid both with increased enantiomeric purity.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (44):

[0041] The Humicola family also includes the following lipolytic enzymes: lipase from Penicillium camembertii (P25234), lipase/phospholipase from Fusarium oxysporum (EP 130064, WO 98/26057), lipase from F. heterosporum (R87979), lysophospholipase from Aspergillus foetidus (W33009), phospholipase A1 from A. oryzae (JP-A 10-155493), lipase from A. olyzae (D85895), lipase/**ferulic acid esterase** from A. niger (Y09330), lipase/**ferulic acid esterase** from A. tubingensis (Y09331), lipase from A. tubingensis (WO 98145453), lysophospholipase from A. niger (WO 98/31790), lipase from F. solani having an isoelectric point of 6.9 and an apparent molecular weight of 30 kDa (WO 96/18729).

Summary of Invention Paragraph - BSTX (154):

[0151] In another preferred embodiment of the present invention the **esterase is ferulic acid esterase** from Aspergillus Oryzae, or acetyl xylan esterase from Aspergillus aculeatus expressed in Aspergillus Oryzae.

Summary of Invention Paragraph - BSTX (192):

[0189] **Ferulic acid esterase** from Aspergillus oryzae,

Summary of Invention Paragraph - BSTX (213):

[0210] **Ferulic acid esterase** from Aspergillus oryzae,

Summary of Invention Paragraph - BSTX (234):

[0231] **Ferulic acid esterase** from Aspergillus oryzae,

Claims Text - CLTX (89):

90. A process according to any one of the preceding claims wherein the **esterase is ferulic acid esterase** from Aspergilus Oryzae, or acetyl xytan esterase from Aspergillus aculeatus expressed in Aspergillus Oryzaee.

Claims Text - CLTX (110):

111. A process according to any one of the preceding claims wherein in relation to Process 1, the enzyme is selected from: Rhizomucor miehei lipase, Humicola lanuginosa lipase, Esperase (Bacillus licheniformis protease), Savinase (Bacillus clausli protease), .alpha.-chymotrypsin from Bovine pancreas, Protease from Pseudomonas putida, e.g. Novozym 180, Proteinase 6 from Aspergillus sp., Flavourzyme from Aspergillus oryzae, Protease 1 (or Aspergillopepsin II) from Aspergillus aculeatus expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease 2 (or

Aspergillopepsin 1) from Aspergillus aculeatus expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Npl protease (or Neutral proteinase I or Fungalysin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Trypsin like protease from Fusarium oxysporum expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Rheozyme, a pectin methyl esterase from Aspergillus aculeatus, Alp. protease (or oryzin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease 2A from Aspergillus oryzae, Pectinex Ultra SP-L from Aspergillus aculeatus, Pectinex BE 3L from Aspergillus niger, Kojizyme 500MG from Aspergillus oryzae, **Ferulic acid esterase** from Aspergillus oiyzae, Acetyl xylan esterase from Aspergillus aculeatus, Shearzyme 500L from Aspergillus aculeatus, Pectinex AFP L-2, Pectinex SMASH, Novozym 188 from Aspergillus niger, Kannase, a variant of Savinase from Bacillus clausii, Cutinase from Humicola insolens, Hydrolytic enzyme mixture obtained from fermentation of Aspergillus oryzae.

Claims Text - CLTX (111):

112. A process according to any one of the preceding claims wherein in relation to Process 1, the enzyme is selected from: Protease 1 (or Aspergillopepsin II) from Aspergillus aculeatus expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease 2 (or Aspergillopepsin i) from Aspergillus aculeatus expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease Npl from Aspergillus aculeatus, Npl protease (or Neutral proteinase I or Fungalysin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Trypsin like protease from Fusarium oxysporum expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Rheozyme, a pectin methyl esterase from Aspergillus aculeatus, Alp. protease (or oryzin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease 2A from Aspergillus oryzae, Pectinex Ultra SP-L from Aspergillus aculeatus, Pectinex BE 3L from Aspergillus niger, Kojizyme 500MG from Aspergillus oryzae, **Ferulic acid esterase** from Aspergillus oryzae, Acetyl xylan esterase from Aspergillus aculeatus, Shearzyme 500L from Aspergillus aculeatus, Pectinex AFP L-2, Pectinex SMASH, Novozym 188 from Aspergillus niger, Kannase, a variant of Savinase from Bacillus clausii, Cutinase from Humicola insolens, Hydrolytic enzyme mixture obtained from fermentation of Aspergillus oryzae.

Claims Text - CLTX (112):

113. A process according to any one of the preceding claims wherein in relation to Process 1, the enzyme is selected from: Protease I (or Aspergillopepsin II) from Aspergillus aculeatus expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease 2 (or Aspergillopepsin I) from Aspergillus aculeatus expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease Npl from Aspergillus aculeatus, Npl protease (or Neutral proteinase I or Fungalysin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Trypsin like protease from Fusarium

oxysporum expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Rheozyme, a pectin methyl esterase from Aspergillus aculeatus, Alp. protease (or oryzin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease 2A from Aspergillus oryzae, Pectinex Ultra SP-L from Aspergillus aculeatus, Pectinex BE 3L from Aspergillus niger, Kojizyme 500MG from Aspergillus oryzae, **Ferulic acid esterase** from Aspergillus oryzae, Acetyl xylan esterase from Aspergillus aculeatus, Shearzyme 500L from Aspergillus aculeatus, Pectinex AFP L-2, Pectinex SMASH, Novozym 188 from Aspergillus niger, Hydrolytic enzyme mixture obtained from fermentation of Aspergillus oryzae.

PGPUB-DOCUMENT-NUMBER: 20030051836

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030051836 A1

TITLE: Enzymatic hydrolysis of a polymer comprising vinyl acetate monomer

PUBLICATION-DATE: March 20, 2003

INVENTOR-INFORMATION:

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Sharyo, Masaki	Matsudo-shi		JP	
Sakaguchi, Hiromichi	Chiba city		JP	
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APPL-NO: 10/ 152300

DATE FILED: May 21, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60294539 20010530 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2001 00813	2001DK-PA 2001 00813	May 21, 2001

US-CL-CURRENT: 162/72, 162/100 , 162/189

ABSTRACT:

The invention relates to the use of certain lipolytic enzymes such as cutinases and lipases in the manufacture of paper and paper products from recycled paper. Examples of such enzymes are derived from strains of Humicola, Candida, Fusarium and Pseudomonas. By use of these enzymes, the problems relating to the so-called "stickies" derived from waste paper are reduced.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims under 35 U.S.C. 119 priority from or the benefit of Danish application No. PA 2001 00813 filed May 21, 2001, and U.S. Provisional No. 60/294,539 filed May 30, 2001, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (129):

[0126] L) **Ferulic acid esterase** from Aspergillus niger.

PGPUB-DOCUMENT-NUMBER: 20030040454

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030040454 A1

TITLE: Cleaning compositions containing plant cell wall degrading enzymes and their use in cleaning methods

PUBLICATION-DATE: February 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cuperus, Roelck A.	Amsterdam		NL	
Herweijer, Margareta A.	Den Haag		NL	
Van Ooijen, Albert J.J.	Voorburg		NL	
Van Schouwen, Dick J.	Vlaardingen		NL	

APPL-NO: 09/ 828374

DATE FILED: April 5, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	PCT/EP95/02380	1995EP-PCT/EP95/02380	June 19, 1995
EP	EP 94201741.9	1994EP-EP 94201741.9	June 17, 1994

US-CL-CURRENT: 510/392, 510/530

ABSTRACT:

Novel cleaning compositions comprising cell wall degrading enzymes are disclosed having pectinases and/or hemicellulases and optionally cellulases. The compositions are particularly suitable for removing stains of vegetable origin, especially from textiles. Although compositions having only one type of such enzymes are part of the invention (excluding cellulases alone), preferred embodiments have a mixture of cell wall degrading enzyme activities to allow for a concerted action against the fibrous mass which usually constitutes a stain of vegetable origin.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (30):

[0030] Endo- and exo-xylanases and accessory enzymes such as glucuronidases, arabinofuranosidases, acetyl xylan **esterase and ferulic** acid or **coumaric acid esterase** have been summarized by Kormelink (1992, Ph.D.-thesis, University of Wageningen, The Netherlands). They are produced by a wide variety of micro-organisms and have varying temperature and pH optima.

Claims Text - CLTX (7):

7. A composition according to any preceding claim wherein the hemicellulase is a xylanase, an arabinofuranosidase, an acetyl xylan **esterase, a glucuronidase, a ferulic acid esterase, a coumaric acid esterase,** an endo-galactanase, a mannanase, a lichenase, an endo- or exo-arabinanase or an exo-galactanase.

Claims Text - CLTX (15):

15. A method according to claim 13 or 14 wherein the composition comprises a xylanase, an arabinofuranosidase, an acetyl xylan **esterase, a glucuronidase, a ferulic acid esterase, a coumaric acid esterase,** a pectin esterase, a pectin lyase, a pectate lyase, an exopolygalacturonase, an endopolygalacturonase, a rhamnogalacturonase, an endoglucanase, an exoglucanase, a .beta.-glucosidase, an endo-galactanase, a mannanase, a lichenase, an endo- or exo-arabinanase or an exogalactanase.

PGPUB-DOCUMENT-NUMBER: 20030035822

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030035822 A1

TITLE: Compositions and methods for enhancing fiber digestion

PUBLICATION-DATE: February 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Tricarico, Juan M.	Lexington	KY	US	
Dawson, Karl A.	Lexington	KY	US	

APPL-NO: 10/ 121476

DATE FILED: April 10, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60282822 20010410 US

US-CL-CURRENT: 424/442, 435/254.2

ABSTRACT:

Methods and enzyme supplements for enhancing fiber digestion in mammals and birds are described. The supplement comprises an effective amount of acetyl esterase, formulated for feeding to animals consuming significant percentages of forages in the diet. The compositions of this invention improve dry matter and neutral detergent fiber disappearance rates, and are useful dietary supplements for improving fiber digestion. The compositions of this invention may be utilized alone or in combination with known exogenous fibrolytic enzyme supplement to improve fiber digestion in mammals and birds. The acetyl esterase advantageously used in the compositions and methods of the present invention is produced by a ruminal isolate of Orpinomyces.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Ser. No. 60/282,822, filed Apr. 10, 2001.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0006] Esterases produced by various anaerobic bacteria and fungi are responsible for removing side groups normally present in plant cell wall

polysaccharides. Other **esterases such as feruloyl and coumaroyl esterases** also break down chemical bonds central to hemicellulose-lignin associations. While not wishing to be bound to any particular theory, it is believed that the actions of esterases increase enzyme accessibility to the backbones of plant cell wall polysaccharides and influence overall plant cell wall structure, thereby improving digestibility. There remains a need in the art for improvements in the economics of agriculture, especially as related to animal feeds. This can be accomplished, at least in part, using alternative enzyme supplements for enhancing fiber digestibility in, e.g., ruminant, equine, porcine, and avian species. There is further a need in the art for alternative enzyme supplements capable of enhancing the efficacy of prior art enzyme supplements for enhancing fiber digestibility in, e.g. ruminant, equine, porcine, and avian species. Ruminant animals of particular economic importance include cattle, sheep, buffaloes and goats. Others include camels, guanaco, llamas, wapiti, antelope, musk oxen, giraffes and others. Improving the efficiency of feed utilization improves the economics of agriculture, thus providing a benefit to the agricultural industry and to society. In addition, captive wild ruminants and other fiber-eating animals can benefit from the present invention.

PGPUB-DOCUMENT-NUMBER: 20030032161

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030032161 A1

TITLE: Esterase enzymes, DNA encoding esterase enzymes and vectors and host cells incorporating same

PUBLICATION-DATE: February 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Borneman, William S.	San Carlos	CA	US	
Bower, Benjamin S.	Pacifica	CA	US	

APPL-NO: 09/ 981430

DATE FILED: October 15, 2001

RELATED-US-APPL-DATA:

child 09981430 A1 20011015

parent division-of 08952445 19971118 US GRANTED

parent-patent 6368833 US

child 08952445 19971118 US

parent continuation-in-part-of 08722713 19960930 US ABANDONED

US-CL-CURRENT: 435/196, 435/252.3 , 435/254.2 , 435/320.1 , 435/325 , 435/6 , 435/69.1 , 536/23.2

ABSTRACT:

A novel DNA is provided which encodes an enzyme having esterolytic activity isolated from Aspergillus. Also provided for is a method of isolating DNA encoding an enzyme having esterolytic activity from organisms which possess such DNA, transformation of the DNA into a suitable host organism, expression of the transformed DNA and the use of the expressed esterase protein in feed as a supplement, in textiles for the finishing of such textiles prior to sale, in starch processing or production of foods such as baked bread.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (4):

[0003] Enzyme hydrolysis of xylan to its monomers requires the participation of several enzymes with different functions. These are classified in two groups based on the nature of the linkages that they cleave. The first group of enzymes is hydrolases (EC 3.2.1) involved in the hydrolysis of the glycosidic bonds of xylan. These include endo-xylanases (EC 3.2.1.8) which randomly dismember the xylan backbone into shorter xylooligosaccharides; .beta.-xylosidase (EC 3.2.1.37) which cleave the xylooligosaccharides in an exo-manner producing xylose; .alpha.-L-arabinofuranosidase (EC 3.2.1.55); and .alpha.-glucoronidase (EC 3.2.1.1) which remove the arabinose and 4-O-methylglucuronic acid substituents, respectively, from the xylan backbone. The second group includes enzymes that hydrolyze the ester linkages (esterase, EC 3.1.1) between xylose units of the xylan polymer and acetyl groups (acetyl xylan esterase, EC 3.1.1.6) or between arabinosyl groups and phenolic moieties such as ferulic acid (**feruloyl esterase) and p-coumaric** acid (**coumaroyl esterase**).

Summary of Invention Paragraph - BSTX (5):

[0004] Faulds et al., reported two forms of **ferulic acid esterase** isolated from Aspergillus niger. The different esterases were distinguished on the basis of molecular weight and substrate specificity (Faulds et al., Biotech. Appl. Biochem., vol. 17, pp. 349-359 (1993)). Brezillon et al. disclosed the existence of at least two **cinnamoyl esterases** which were believed to be distinct from the **ferulic acid esterases** shown in the prior art (Brezillon et al., Appl. Microb. Biotechnol., vol. 45, pp. 371-376 (1996)). A **ferulic acid esterase** called FAE-III was isolated from Aspergillus niger CBS 120.49 and shown to act together with xylanase to eliminate nearly all of the ferulic acid and low molecular mass xylooligosaccharides in a wheat bran preparation; ferulic acid was also removed without the addition of xylanase, albeit at a lower level. Faulds et al. further isolated and partially characterized FAE-III from Aspergillus niger CBS120.49 grown on oat spelt xylan (Faulds et al., Microbiology, vol. 140, pp. 779-787 (1994)) and showed it to have a pI of 3.3, a molecular weight of 36 kD (SDS-PAGE) and 14.5 kD (Gel Filtration method), a pH optimum of 5 and a temperature optimum of 55-60 C; microcrystalline cellulose binding was also detected. The authors theorized that FAE-II may be a proteolytically modified FAE-III. Recently, the various known **ferulic acid esterases** derived from Aspergillus niger have been distinguished based on their distinct substrate specificity and it was noted that FAE-II and FAE-III were unable to release ferulic acid from sugar beet pulp (Brezillon et al., supra).

Detail Description Paragraph - DETX (2):

[0022] "Esterase" or "esterolytic activity" means a protein or peptide which exhibits esterolytic activity, for example, those enzymes having catalytic activity as defined in enzyme classification EC 3.1.1. Esterolytic activity may be shown by the ability of an enzyme or peptide to cleave ester linkages, for example, feruloyl, coumaroyl or acetyl xylan groups, from organic compounds in which they are known to exist, e.g., primary and secondary cell walls. Preferably, the esterase comprises an esterolytic activity which cleaves the ester linkage of phenolic esters such as:

[5-O-((E)-feruloyl)-.alpha.-L-arabinofuranosyl] (1.fwdarw.3)-O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-D-xylopyranose (also known as FAXX); [5-O-((E)-feruloyl)-.alpha.-L-arabinofuranosyl] (1.fwdarw.3)-O-.beta.-D-xylopyranose (also known as FAX); O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-O-[5-O-((E)-feruloyl)-.alpha.-arabinofuranosyl-(1.fwdarw.3)]-O-.beta.D-xylopyranosyl-(1.fwdarw.4)-D-xylopyran- ose (also known as FAXXX); [5-O-((E)-p-coumaroyl)-.alpha.-L-arabinofuranos- yl] (1.fwdarw.3)-O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-D-xylopyranose (also known as PAXX); [5-O-((E)-p-coumaroyl)-.alpha.-L-arabinofuranosyl] (1.fwdarw.3)-O-.beta.-D-xylopyranose (also known as PAX); O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-O-[5-O-((E)-p-coumaroyl)-.alpha.-arabinofuranosyl-(1.fwdarw.3)]-O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-D-xylopyranose (also known as PAXXX) and other ester linked phenolic oligosaccharides as are known in the art. Such **esterases are generally referred to as ferulic acid esterase** (FAE) or enzymes having **feruloyl esterase** activity. It has surprisingly been discovered that an **esterase having ferulic acid esterase** activity which may be purified from Aspergillus niger, as described herein, and having an amino acid sequence as shown in FIG. 5, further has activity on sugar beet pulp and also proteolytic and lipolytic activity. Thus, according to a particularly preferred embodiment of the present invention, an esterase and/or a DNA encoding that esterase is provided which esterase also has lipolytic and/or proteolytic activity. Accordingly, the esterase of the invention having measurably significant esterolytic activity on feruloyl and coumaroyl esters also has proteolytic and lipolytic activity.

Detail Description Paragraph - DETX (17):

[0035] Purification and Isolation of Peptides Comprising **Ferulic Acid Esterase** Activity and Design of Degenerate DNA Fragments for PCR

Detail Description Table CWU - DETL (8):

8TABLE 3 Release of ferulic acid from sugar beet pulp with **ferulic acid esterase** Ferulic acid released from sugar beet pulp Enzyme 12 hrs 24 hrs treatment .mu.g % .mu.g % FAE 15.3 2.7 26.2 4.6 Xylanase 0.5 0.1 0.6 0.1 FAE + 27.1 4.8 49.7 8.7 Xylanase Buffer Control 0.2 0.04 0.2 0.04 Inactivated 0.2 0.03 0.2 0.04 FAE

Claims Text - CLTX (6):

5. The DNA of claim 4, wherein said DNA is derived from Aspergillus and comprises a **feruloyl esterase**.

PGPUB-DOCUMENT-NUMBER: 20030024009

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030024009 A1

TITLE: Manipulation of the phenolic acid content and digestibility of plant cell walls by targeted expression of genes encoding cell wall degrading enzymes

PUBLICATION-DATE: January 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Langdon, Timothy	Aberystwyth		GB	
Morris, Phillip	Aberystwyth		GB	

APPL-NO: 09/ 991209

DATE FILED: November 16, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60249608 20001117 US

US-CL-CURRENT: 800/281, 435/196 , 435/320.1 , 435/419 , 435/69.1 , 536/23.2 , 800/320.3

ABSTRACT:

Described herein are methods to enhance the production of more highly fermentable carbohydrates in plants, especially forage grasses. The invention provides for transgenic plants transformed with expression vectors containing a DNA sequence encoding **ferulic acid esterase** I from Aspergillus, preferably A. niger. The expression vectors may optionally comprise a DNA sequence encoding xylanase from Trichoderma, preferably T. reesei. Expression of the enzyme(s) is targeted to specific cellular compartments, in specific tissues and under specific environmental conditions. Uses of this invention include, but are not limited to, forage with improved digestibility for livestock, and enhanced biomass conversion.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] Pursuant to 35 U.S.C. .sctn.119(e), the present application claims benefit of and priority to U.S. Ser. No. 60/249,608, entitled "MANIPULATION OF THE PHENOLIC ACID CONTENT AND DIGESTIBILITY OF FORAGE GRASS CELL WALLS BY
TARGETED EXPRESSION OF A **FERULIC ACID ESTERASE** GENE", filed Nov. 17, 2000, by Morris et al.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

Described herein are methods to enhance the production of more highly fermentable carbohydrates in plants, especially forage grasses. The invention provides for transgenic plants transformed with expression vectors containing a DNA sequence encoding **ferulic acid esterase** I from Aspergillus, preferably A. niger. The expression vectors may optionally comprise a DNA sequence encoding xylanase from Trichoderma, preferably T. reesei. Expression of the enzyme(s) is targeted to specific cellular compartments, in specific tissues and under specific environmental conditions. Uses of this invention include, but are not limited to, forage with improved digestibility for livestock, and enhanced biomass conversion.

Cross Reference to Related Applications Paragraph - CRTX (1):

[0001] Pursuant to 35 U.S.C. .sctn.119(e), the present application claims benefit of and priority to U.S. Ser. No. 60/249,608, entitled "MANIPULATION OF THE PHENOLIC ACID CONTENT AND DIGESTIBILITY OF FORAGE GRASS CELL WALLS BY
TARGETED EXPRESSION OF A **FERULIC ACID ESTERASE** GENE", filed Nov. 17, 2000, by Morris et al.

Summary of Invention Paragraph - BSTX (10):

[0008] In one embodiment, the cell wall degrading enzyme is selected from the group consisting of **ferulic acid esterase,** xylanase, xylosidase, cellulase, endoglucanase, and cellbiohydrolase. In a preferred embodiment cell wall degrading enzyme is derived from a fungal source. In a more preferred embodiment, the fungal **ferulic acid esterase is an Aspergillus ferulic acid esterase,** preferably A. niger. In another embodiment the xylanase is derived from Trichoderma, preferably T. reesei.

Summary of Invention Paragraph - BSTX (12):

[0010] Further provided herein is a method of controlling the level of phenolic acids in plant cell walls of a transgenic plant. The method, in one embodiment, comprises introducing to a plant cell an expression cassette comprising a DNA sequence encoding at least one cell wall degrading enzyme, preferably a **ferulic acid esterase**.

Brief Description of Drawings Paragraph - DRTX (2):

[0012] FIG. 1 illustrates a restriction map of a DNA fragment containing the gene encoding the 38 kd **ferulic acid esterase**.

Brief Description of Drawings Paragraph - DRTX (3):

[0013] FIGS. 2A-E illustrate the complete DNA (SEQ. ID NO:_____), with highlighting to point out the signal sequence, intron and various restriction endonuclease sites, and amino acid sequence (SEQ. ID. NO:_____) corresponding to the gene encoding the 38 kD **ferulic acid esterase** isolated from Aspergillus niger.

Brief Description of Drawings Paragraph - DRTX (5):

[0015] FIG. 4 illustrates the construction of the intronless **ferulic acid esterase** isolated from Aspergillus niger.

Detail Description Paragraph - DETX (24):

[0087] The term "plasmid" refers to a circular double stranded DNA molecule which comprises the coding sequence of interest, regulatory elements, a selection marker and optionally an amplification marker. A plasmid can transform prokaryotic cells or transfect eukaryotic cells. An "expression cassette" means a portion of a plasmid (or the entire plasmid) containing the regulatory elements desired for transcription, translation and/or expression and the coding region of a polynucleotide. A plasmid may contain one or more expression cassettes. If multiple expression cassettes are introduced into a plant, they can be introduced simultaneously or at different times. If simultaneous introduction is desired, the expression cassettes can be on one plasmid or more. Typically, an expression cassette comprises a promoter, poly A+ tail, and signal sequences that target the expressed polypeptide to a specific region of a cell or to be secreted, if desired. Examples of signal sequences that "target expression" of **ferulic acid esterase** include sequences located upstream of the FAE coding sequence. The polynucleotide that encodes the signal sequence is found preferably within the 100 nucleotides "upstream" (in the 5' direction) from the initiation codon (AUG). More preferably, the polynucleotide that encodes the signal sequence is found within the 50 nucleotides upstream from the initiation codon. Many different cellular organelles are targeted by the signal sequences used in this invention. The organelles include, but are not limited to, vacuoles, Golgi apparati, endoplasmic reticula, and apoplasts. In addition to upstream signal sequences, the expression cassette of this invention may include a polynucleotide that encodes a signal sequence at the 3' end. These signal sequences include, but are not limted to stop codons and the KDEL sequence. In addition to KDEL, other similar sequences are contemplated by this invention, including but not limited to RDEL. In addition to a KDEL sequence, a signal sequence can include a linker to a KDEL sequence. A linker is an extension of the reading frame of the encoding polynucleotide to the signal sequence. Preferably, the polynucleotide encoding the signal sequence is directly downstream from the coding sequence, more preferably less than 100 base pairs from the stop codon, more preferably less than 20 base pairs from the stop codon.

Detail Description Paragraph - DETX (26):

[0089] The term "polypeptide," "peptide," and "protein" are used interchangeably and refer to amino acids connected by peptide bonds. Polypeptides can be entire proteins or portions thereof. For Example. a FAE1 polypeptide may refer to the entire FAE1 protein or fragments of the FAE1 protein. A "**ferulic acid esterase** with an altered glycosylation site" refers to a FAE protein wherein a mutation has changed the glycosylation pattern of the protein. Mutations that effect such changes are well known in the art and include, but are not limited to, amino acid substitutions, and mutations in the proteins of the Golgi apparatus and endoplasmic reticulum that effect glycosylation of proteins.

Detail Description Paragraph - DETX (37):

[0100] **Feruloyl esterase** activity has been detected in several fungal species including, anaerobic gut fungi, yeasts, actinomycetes, and a few fiber-degrading ruminal bacteria, which enables them to de-esterify arabinoxylans and pectins.

Detail Description Paragraph - DETX (38):

[0101] Two **ferulic acid esterases** (FAE), distinguished on the basis of molecular weight and substrate specificity, have been isolated from Aspergillus niger and have been shown to quantitatively hydrolyze ferulic acid and release dehydrodiferulate dimers from plant cell walls. Furthermore, FAE has been observed to act synergistically with xylanase to release ferulic acid from plant cell walls at a higher rate. Recently, a **ferulic acid esterase** (FAE) gene has been cloned from Aspergillus niger (Michelson, et. al. European Patent Application No. 9510370.1). The inventors have found the recombinant enzyme releases ferulic acid and diferulate dimers from grass cell walls in a concentration dependent manner and that this enzyme is stable at 30.degree. C. pH 5.0 in the presence of substrate and has a half life of 61 h at 30.degree. C. in the presence of vacuolar extracts (pH 4.6) of grass cells. This gene was, therefore, a candidate for targeted and indicible expression of FAE in grasses (e.g., Lolium multiflorum).

Detail Description Paragraph - DETX (39):

[0102] The present invention provides for methods of changing the cell wall structure of transgenic plants and therefore, making them more digestible. The method comprises introducing a **ferulic acid esterase** coding sequence into the cells of a plant. Operably linked to the coding seqeunce is a promoter that can be either constitutive or inducible and signal sequences that serve to target expression of the coding sequence in the desired organelle in the desired cell of the plant. The signal sequences can be either or both N terminal or C terminal sequences.

Detail Description Paragraph - DETX (48):

[0111] Appropriate primers and probes for identifying **ferulic acid esterase**-specific genes, as well as xylanase sequences, from fungi and plant tissues are generated from comparisons of the sequences provided herein. For a general overview of PCR see PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS,
(Innis, M, Gelfand, D., Sninsky, J. and White, T., eds.), Academic Press, San Diego (1990). Reaction components are typically: 10 mM Tris-HCl, pH 8.3, 50 mM potassium chloride, 1.5 mM magnesium chloride, 0.001% gelatin, 200 .mu.M dATP, 200 .mu.M dCTP, 200 .mu.M dGTP, 200 .mu.M dTTP, 0.4 .mu.M primers, and 100 units per mL Taq polymerase. Program: 96.degree. C. for 3 min., 30 cycles of 96.degree. C. for 45 sec., 50.degree. C. for 60 sec., 72.degree. C. for 60 sec, followed by 72.degree. C. for 5 min.

Detail Description Paragraph - DETX (50):

[0113] Suitable sources for the **ferulic acid esterase** used in this invention include but are not limited to, Neurospora crassa, Aspergillus spp. and specifically, Aspergillus niger. The xylanase used in this invention can be derived from any suitable source including, but not limited to, Trichoderma reesei and Aspergillus spp.

Detail Description Paragraph - DETX (78):

[0140] Ferulic and other hydroxycinnamic acids are ester linked to arabinosyl residues in arabinoxylans, and play a key role in crosslinking xylans to liginin, resulting in less degradable cell walls. **Ferulic acid esterase** (FAE) can release both monomeric and dimeric ferulic acid (FA) from arabinoxylans making the cell wall more susceptible to further enzymatic attack. Transgenic plants have been produced expressing an FAE gene following microprojectile bombardment of cell cultures. Measurements of the level of FAE activity from different vectors targeting FAE to the vacuole, ER and apoplast under constitutive or inducible (heat shock) promoters shows that at least for constitutive expression of vacuolar targeted FAE, the activity was highest in young leaves and increased along the leaf lamina. We also show that FAE expression results in release of monomeric and dimeric FA from cell walls on cell death and this was enhanced several fold by the addition of xylanase. An effect of FAE expression on the monomeric and dimeric cell wall ester linked ferulate content in comparison to control (non-transformed) plants is seen. Generally, the lower the levels of monomers and, in particular, dimers of hydroxycinnamic acids in leaves, the higher the digestibility and/or availability of complex carbohydrates for conversion.

Claims Text - CLTX (2):

1. A transgenic plant comprising an expression cassette comprising a promoter operably linked to a **ferulic acid esterase** encoding polynucleotide.

Claims Text - CLTX (5):

4. The plant of claim 3, wherein the polynucleotide encodes a **ferulic acid esterase** with an altered glycosylation site.

Claims Text - CLTX (6):

5. The plant of claim 3, wherein the polynucleotide encodes a **ferulic acid esterase** with a substitution so that glycosylation is altered.

Claims Text - CLTX (9):

8. The plant of claim 1, wherein the introduction of the **ferulic acid esterase** polynucleotide into the plant is by sexual reproduction.

Claims Text - CLTX (15):

14. The plant of claim 13, wherein the polynucleotide sequence is upstream of the N-terminus of the **ferulic acid esterase** polynucleotide.

Claims Text - CLTX (24):

23. The plant of claim 22, wherein the signal sequence is from Aspergillus niger **ferulic acid esterase**.

Claims Text - CLTX (26):

25. The plant of claim 13, wherein the polynucleotide sequence is downstream of the C-terminus of the **ferulic acid esterase** polynucleotide

Claims Text - CLTX (29):

28. The plant of claim 25, wherein the polynucleotide sequence is an extension of the **ferulic acid esterase** reading frame to provide a linker to KDEL.

Claims Text - CLTX (35):

34. A method of controlling the level of phenolic acids in plant cell walls of a transgenic plant, the method comprising introducing into the plant an expression cassette comprising a promoter operably linked to a **ferulic acid esterase** encoding polynucleotide.

Claims Text - CLTX (38):

37. The method of claim 36, wherein the polynucleotide encodes the **ferulic**

acid esterase with an altered glycosylation site.

Claims Text - CLTX (39):

38. The method of claim 36, wherein the polynucleotide encodes the **ferulic acid esterase** with a substitution such that glycosylation is altered.

Claims Text - CLTX (43):

42. The method of claim 34, wherein the introduction of the **ferulic acid esterase** polynucleotide into the plant is by transformation of cell cultures.

Claims Text - CLTX (45):

44. The method of claim 34 wherein the **ferulic acid esterase** polynucleotide is introduced into the plant by sexual reproduction.

Claims Text - CLTX (55):

54. The method of claim 53, wherein the polynucleotide sequence is upstream of the N-terminus of the **ferulic acid esterase** polynucleotide.

Claims Text - CLTX (64):

63. The method of claim 62, wherein the signal sequence is from Aspergillus niger **ferulic acid esterase**.

Claims Text - CLTX (66):

65. The method of claim 53, wherein the polynucleotide sequence is downstream of the C-terminus of the **ferulic acid esterase** polynucleotide

Claims Text - CLTX (69):

68. The method of claim 65, wherein the polynucleotide sequence is an extension of the **ferulic acid esterase** reading frame to provide a linker to KDEL.

PGPUB-DOCUMENT-NUMBER: 20030017575

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030017575 A1

TITLE: Cell-wall degrading enzyme variants

PUBLICATION-DATE: January 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Andersen, Carsten	Vaerlose		DK	
Schulein, Martin	Copenhagen		DK	
Dela, Hanne	Copenhagen		DK	
Frandsen, Torben Peter	Frederiksberg		DK	

APPL-NO: 09/ 910505

DATE FILED: July 19, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60290724 20010514 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 01117	2000DK-PA 2000 01117	July 19, 2000
DK	PA 2001 00705	2001DK-PA 2001 00705	May 4, 2001
DK	PA 2001 00734	2001DK-PA 2001 00734	May 10, 2001

US-CL-CURRENT: 435/232, 435/101 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

A variant of a cell-wall degrading enzyme having a beta-helix structure, which variant holds at least one substituent in a position determined by identifying all residues potentially belonging to a stack; characterizing the stack as interior or exterior; characterizing the stack as polar, hydrophobic or aromatic/heteroaromatic based on the dominating characteristics of the parent or wild-type enzyme stack residues and/or its orientation relative to the beta-helix (interior or exterior); optimizing all stack positions of a stack either to hydrophobic aliphatic amino acids, hydrophobic aromatic or polar amino acids by allowing mutations within one or all positions to amino acids belonging to one of these groups; measuring thermostability of the variants by DSC or an application-related assay such as a Pad-Steam application test; and selecting the stabilized variants. Variant of a wild-type parent pectate lyase (EC 4.2.2.2) having the conserved amino acid residues D111, D141 or E141, D145, K165, R194 and R199 when aligned with the pectate lyase comprising the amino acid sequence of SEQ ID NO: 2 are preferred.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims, under 35 U.S.C. 119, priority of Danish application nos. PA 2000 01117, filed July 19, 2000, PA 2001 00705, filed May 4, 2001, and PA 2001 00734, filed May 10, 2001, and the benefit of U.S. provisional application No. 60/290724, filed May 14, 2001, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (42):

[0041] Endo- and exo-xylanases and accessory enzymes such as glucuronidases, arabinofuranosidases, acetyl xylan **esterase and ferulic** acid or **coumaric acid esterase** have been summarized by Kormelink (1992, Ph.D.-thesis, University of Wageningen, The Netherlands). They are produced by a wide variety of micro-organisms and have varying temperature and pH optima.

PGPUB-DOCUMENT-NUMBER: 20030017221

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030017221 A1

TITLE: Enzymatically catalyzed hydrolysis of corn fiber and products obtained from enzymatically hydrolyzed corn fiber

PUBLICATION-DATE: January 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Antrim, Richard L.	Solon	IA	US	

APPL-NO: 10/ 109210

DATE FILED: March 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60279377 20010328 US

non-provisional-of-provisional 60283421 20010412 US

US-CL-CURRENT: 424/750, 435/101

ABSTRACT:

Disclosed is a method for treating a grain fiber, in particular corn fiber. In accordance with the disclosed subject matter, the fiber is subjected to alkaline hydrolysis in the presence of a hemicellulose ferulate esterase in an aqueous medium. The hemicellulose ferulate esterase is present in an amount effective to catalyze alkaline hydrolysis, and the alkaline hydrolysis is performed under conditions suitable to permit the hemicellulose ferulate esterase to catalyze alkaline hydrolysis. The initial pH of the aqueous medium is alkaline but is sufficiently low to permit the enzyme to have catalytic activity. Preferably, the pH is in the range from 8.0 to 9.5. From the product mixture thus formed, fiber oil, cellulose, hemicellulose, and other materials may be obtained.

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims priority to prior U.S. provisional application Serial No. 60/279,377, filed Mar. 28, 2001. This application also claims priority to prior U.S. provisional application Serial No. 60/238,421, filed Apr. 12, 2001. Both prior applications are hereby incorporated by reference in their entities.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (18):

[0015] The methods of the present invention preferably are performed with various catalyzing enzymes. In the highly preferred embodiment of the invention, a hemicellulose ferulate esterase is employed. Hemicellulose ferulate esterases, which contain one or more domains that catalyze the cleavage of ferulic acid esters, are a class of enzymes known in the art, and the hemicellulose ferulate esterase used in conjunction with the invention may be derived from a microbe using conventional techniques. For instance, it is known to isolate **ferulic acid esterases** from Aspergillus niger. Preferably, the microbe is obtained from an ecological niche associated with conditions under which cellulosic materials such as cellulose and hemicellulose are decomposed. It is believed that certain **ferulic acid esterases** will be specific to catalysis of the hydrolysis of hemicellulose, as opposed to esters that compose the corn fiber oil. The invention further contemplates the use of a coumeric acid esterase, which is a known class of enzymes that contains one or more domains that catalyze the cleavage of coumeric acid esters. Coumeric acid esterase may be isolated from microbes (e.g. the Aspergillus family) in accordance with known techniques. The hemicellulose ferulate esterase and coumeric acid esterase may be considered together as "hemicellulose liberating enzymes."

PGPUB-DOCUMENT-NUMBER: 20030008899

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030008899 A1

TITLE: Heterocyclic derivatives

PUBLICATION-DATE: January 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Orlandi, Alessandra	Lainate		IT	

APPL-NO: 10/ 148434

DATE FILED: May 29, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	9929037.1	1999GB-9929037.1	December 8, 1999

PCT-DATA:
APPL-NO: PCT/EP00/12335
DATE-FILED: Dec 7, 2000
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/314, 546/167

ABSTRACT:

The present invention relates to a novel salt of enantiomer A of 7-chloro-4-(2-oxo-1-phenyl-3-pyrrolidinylidene) 1,2,3,4-tetrahydro-2-quinoline carboxylic acid or a solvate thereof, to processes for its preparation, to pharmaceutical compositions containing it and to its use in therapy and in particularly its use as medicine for antagonising the effects of excitatory amino acids upon the NMDA receptor complex.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (45):

[0045] In a preferred embodiment, the enantiomer A of the compound of formula (I) may be prepared by stereoselective enzymatic hydrolysis of compounds of formula (II) with **ferulic acid esterase** in a pure form. 2

Summary of Invention Paragraph - BSTX (50):

[0050] The stereoselective enzymatic hydrolysis of compounds of formula (II) with **ferulic acid esterase** in a pure form is novel and represents a further aspect of the invention.

Summary of Invention Paragraph - BSTX (51):

[0051] The invention also extends to the meglumine salt of enantiomer A of 7-chloro-4-(2-oxo-1-phenyl-3-pyrrolidinylidene)-1,2,3,4-tetrahydro-2-quinolinecarboxylic acid of formula (I) or a solvate thereof when prepared from the enantiomer A of formula (I) which has been obtained by stereoselective enzymatic hydrolysis of compounds of formula (II) with **ferulic acid esterase** in a pure form.

Detail Description Paragraph - DETX (12):

[0098] To a warmed at 35.degree. C. 0.1M Sodium citrate buffer obtained by mixing a 0.1M aqueous solution (412 ml) of citric acid and a 0.1M aqueous solution (196 ml) of trisodic citrate dihydrate into a jacketed reactor, an aqueous solution (Conc=40 mg/ml) of the enzyme **ferulic acid esterase** (19.6 ml) and dimethyl sulfoxide (98 ml) were added. To the resulting solution, a solution of Intermediate 4 (49 g) in dimethyl sulfoxide (270 ml) was added. Then the mixture was stirred at 37-38.degree. C. for 24 hrs.

PGPUB-DOCUMENT-NUMBER: 20030008361

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030008361 A1

TITLE: Process for the preparation of substituted 3-phenyl-propanoic acid esters and substituted 3-phenyl-propanoic acids

PUBLICATION-DATE: January 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ebdrup, Soren	Kobenhavn O		DK	
Deussen, Heinz-Josef W.	Soborg		DK	
Zundel, Magali	Soborg		DK	
Bury, Paul Stanley	Kobenhavn NV		DK	

APPL-NO: 10/ 132428

DATE FILED: April 24, 2002

RELATED-US-APPL-DATA:

child 10132428 A1 20020424

parent continuation-of 09633613 20000807 US ABANDONED

non-provisional-of-provisional 60148643 19990812 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 1999 01101	1999DK-PA 1999 01101	August 5, 1999

US-CL-CURRENT: 435/135, 435/136

ABSTRACT:

The present invention relates to a process comprising hydrolysis or trans esterification of one of the two enantiomeric forms of a racemic or enantiomerically enriched ester of formula I or IV by a higher rate than the other by an enzyme to give an ester and a acid (III) or two different esters (V) and (VI) with different R groups both with increased enantiomeric purity and a esterification process of a racemic or enantiomerically enriched acid (VII) by an enzyme to give an ester and an acid both with increased enantiomeric purity.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (48):

[0045] The Humicola family also includes the following lipolytic enzymes: lipase from Penicillium camembertii (P25234), lipase/phospholipase from Fusarium oxysporum (EP 130064, WO 98/26057), lipase from F. heterosporum (R87979), lysophospholipase from Aspergillus foetidus (W33009), phospholipase AI from A. oryzae (JP-A 10-155493), lipase from A. oryzae (D85895), lipase/**ferulic acid esterase** from A. niger (Y09330), lipase/**ferulic acid esterase** from A. tubingensis (Y09331), lipase from A. tubingensis (WO 98/45453), lysophospholipase from A. niger (WO 98/31790), lipase from F. solanii having an isoelectric point of 6.9 and an apparent molecular weight of 30 kDa (WO 96/18729).

Summary of Invention Paragraph - BSTX (263):

[0260] In another preferred embodiment of the present invention the **esterase is ferulic acid esterase** from Aspergillus Oryzae, or acetyl xylan esterase from Aspergillus aculeatus expressed in Aspergillus Oryzae.

Summary of Invention Paragraph - BSTX (301):

[0298] **Ferulic acid esterase** from Aspergillus oryzae,

Summary of Invention Paragraph - BSTX (322):

[0319] **Ferulic acid esterase** from Aspergillus oryzae,

Summary of Invention Paragraph - BSTX (343):

[0340] **Ferulic acid esterase** from Aspergillus oryzae,

Claims Text - CLTX (138):

138. A process according to any one of the preceding claims wherein the **esterase is ferulic acid esterase** from Aspergillus Oryzae, or acetyl xylan esterase from Aspergillus aculeatus expressed in Aspergillus Oryzae.

Claims Text - CLTX (159):

159. A process according to any one of the preceding claims wherein in relation to Process 1, the enzyme is selected from: Rhizomucor miehei lipase, Humicola lanuginosa lipase, Esperase (Bacillus licheniformis protease), Savinase (Bacillus clausii protease), .alpha.-chymotrypsin from Bovine pancreas, Protease from Pseudomonas putida, e.g. Novozym 180, Proteinase 6 from Aspergillus sp., Flavourzyme from Aspergillus oryzae, Protease 1 (or Aspergillopepsin II) from Aspergillus aculeatus expressed in Aspergillus oryzae

also containing secreted enzymes from Aspergillus oryzae, Protease 2 (or Aspergillopepsin I) from Aspergillus aculeatus expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Npl protease (or Neutral proteinase I or Fungalysin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Trypsin like protease from Fusarium oxysporum expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Rheozyme, a pectin methyl esterase from Aspergillus aculeatus, Alp. protease (or oryzin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease 2A from Aspergillus oryzae, Pectinex Ultra SP-L from Aspergillus aculeatus, Pectinex BE 3L from Aspergillus niger, Kojizyme 500MG from Aspergillus oryzae, **Ferulic acid esterase** from Aspergillus oryzae, Acetyl xylan esterase from Aspergillus aculeatus, Shearzyme 500L from Aspergillus aculeatus, Pectinex AFP L-2, Pectinex SMASH, Novozym 188 from Aspergillus niger, Kannase, a variant of Savinase from Bacillus clausii, Cutinase from Humicola insolens, Hydrolytic enzyme mixture obtained from fermentation of Aspergillus oryzae.

Claims Text - CLTX (160):

160. A process according to any one of the preceding claims wherein in relation to Process 1, the enzyme is selected from: Protease 1 (or Aspergillopepsin II) from Aspergillus aculeatus expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease 2 (or Aspergillopepsin I) from Aspergillus aculeatus expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease Npl from Aspergillus aculeatus, Npl protease (or Neutral proteinase I or Fungalysin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Trypsin like protease from Fusarium oxysporum expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Rheozyme, a pectin methyl esterase from Aspergillus aculeatus, Alp. protease (or oryzin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease 2A from Aspergillus oryzae, Pectinex Ultra SP-L from Aspergillus aculeatus, Pectinex BE 3L from Aspergillus niger, Kojizyme 500MG from Aspergillus oryzae, **Ferulic acid esterase** from Aspergillus oryzae, Acetyl xylan esterase from Aspergillus aculeatus, Shearzyme 500L from Aspergillus aculeatus, Pectinex AFP L-2, Pectinex SMASH, Novozym 188 from Aspergillus niger, Kannase, a variant of Savinase from Bacillus clausii, Cutinase from Humicola insolens, Hydrolytic enzyme mixture obtained from fermentation of Aspergillus oryzae.

Claims Text - CLTX (161):

161. A process according to any one of the preceding claims wherein in relation to Process 1, the enzyme is selected from: Protease 1 (or Aspergillopepsin II) from Aspergillus aculeatus expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease 2 (or Aspergillopepsin I) from Aspergillus aculeatus expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease Npl from Aspergillus aculeatus, Npl protease (or Neutral proteinase I or Fungalysin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing

secreted enzymes from Aspergillus oryzae, Trypsin like protease from Fusarium oxysporum expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Rheozyme, a pectin methyl esterase from Aspergillus aculeatus, Alp. protease (or oryzin) from Aspergillus oryzae expressed in Aspergillus oryzae also containing secreted enzymes from Aspergillus oryzae, Protease 2A from Aspergillus oryzae, Pectinex Ultra SP-L from Aspergillus aculeatus, Pectinex BE 3L from Aspergillus niger, Kojizyme 500MG from Aspergillus oryzae, **Ferulic acid esterase** from Aspergillus oryzae, Acetyl xylan esterase from Aspergillus aculeatus, Shearzyme 500L from Aspergillus aculeatus, Pectinex AFP L-2, Pectinex SMASH, Novozym 188 from Aspergillus niger, Hydrolytic enzyme mixture obtained from fermentation of Aspergillus oryzae.

PGPUB-DOCUMENT-NUMBER: 20020164399

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020164399 A1

TITLE: Process for the production of alcoholic beverages using maltseed

PUBLICATION-DATE: November 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Souppe, Jerome	Wasquehal		FR	
Beudeker, Robert Franciscus	KC Den Hoorn		NL	

APPL-NO: 09/ 970616

DATE FILED: October 4, 2001

RELATED-US-APPL-DATA:

child 09970616 A1 20011004

parent continuation-of 09230590 19990428 US PATENTED

child 09230590 19990428 US

parent a-371-of-international PCT/EP97/04016 19970723 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	96202195.2	1996EP-96202195.2	August 5, 1996

US-CL-CURRENT: 426/11, 426/12 , 426/16

ABSTRACT:

The invention relates to a process for the production of alcoholic beverages such as beer or whiskey with a mixture of enzymes comprising an endo-.beta.(1,4)-xylanase, an arabinofuranosidase, an alpha-amylase, an endo-protease and a .beta.-(1,3; 1,4)-glucanase, and optionally, a saccharifying amylase and/or an exopeptidase. Preferable are mixtures in which the enzymes which are necessary in the brewing process are provided by transgenic seeds. Only a minor amount of malt may be necessary for flavour and color.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (30):

[0026] Hemicellulolytic enzymes comprise enzymes like .beta.-1,3-1,4-glucanase, xylanase, endo-arabinanase, arabinofuranosidase, arabinoxylanase, arabinogalactanase, **ferulic acid esterase**.

PGPUB-DOCUMENT-NUMBER: 20020157798

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020157798 A1

TITLE: Neutral deinking with a deinking composition comprising a lipase and a fatty acid ester

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Franks, Neal	Raleigh	NC	US	
Page, Kelly W.	Bailey	NC	US	

APPL-NO: 10/ 050489

DATE FILED: January 16, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60261784 20010116 US

US-CL-CURRENT: 162/5, 162/72 , 162/75 , 162/76

ABSTRACT:

The present invention relates to methods for deinking wastepaper by pulping wastepaper at a pH between 4 and 8.5 in the presence of deinking agents comprising a lipase and a fatty acid ester and removing the thereby dislodged ink particles.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application no. 60/261,784, filed on Jan. 16, 2001, the contents of which are hereby incorporated by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (3):

[0012] The lipase enzyme to be used in the present invention is one that can hydrolyze ester bonds. Such enzymes include, for example, lipases, such as triacylglycerol lipase (EC 3.1.1.3), lipoprotein lipase (EC 3.1.1.34), monoglyceride lipase (EC 3.1.1.23), lysophospholipase, **ferulic acid esterase** and esterase (EC 3.1.1.1, EC 3.1.1.2). The numbers in parentheses are the systematic numbers assigned by the Enzyme Commission of the International Union

of Biochemistry in accordance with the type of the enzymatic reactivity of the enzyme.

PGPUB-DOCUMENT-NUMBER: 20020146484

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020146484 A1

TITLE: Concentrated spent fermentation beer of saccharopolyspora erythraea activated by an enzyme mixture as a nutitional feed supplement

PUBLICATION-DATE: October 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fidler, Daniel J.	Gurnee	IL	US	
Lampel, Jay Sanford	Mundelein	IL	US	
Weyant, Daniel B.	Winthrop Harbor	IL	US	

APPL-NO: 10/ 036908

DATE FILED: December 21, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60259163 20010102 US

US-CL-CURRENT: 426/53

ABSTRACT:

A feed additive composition which contains enzyme-treated, concentrated spent fermentation beer of Saccharopolyspora erythraea; a method for making a feed additive containing enzyme-treated concentrated spent fermentation beer of Saccharopolyspora erythraea; a process for improving the nutritive value of spent fermentation beer of Saccharopolyspora erythraea by enzymatic treatment; a method for feeding livestock with a feed containing an enzyme-treated, concentrated spent fermentation beer of Saccharopolyspora erythraea additive and a process for improving poultry feed conversion, breast meat yield and intestinal strength by feeding a feed containing an enzyme-treated, concentrated spent fermentation beer of Saccharopolyspora erythraea additive are disclosed. Preferably, the spent fermentation beer of Saccharopolyspora erythraea is treated with a cellulase and at least one glycosidase, and then concentrated.

[0001] This application claims priority to the provisional application Serial No. 60/259,163 filed on Jan. 2, 2001.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (10):

[0009] Some enzymes have been clearly recognized in the marketplace for their value as additives in animal feeds: xylanse, .beta.-glucanase, enzymes that cleave phosphorus from phytic acid, hemicellulates (as disclosed in U.S. Pat. No. 6,162,473), **ferulic acid esterase** (as disclosed in U.S. Pat. No.6,143,543) and mannanase. In addition to enzyme feed additives, small molecules such as aminocarboxylic acid derivatives as disclosed in U.S. Pat. No. 6,166,086 are also useful, and marine mammals treated with proteolytic enzymes have also been disclosed (U.S. Pat. No. 6,153,251). Fermentation products are also known as feed additives, such as a fermented formula feed obtainable from mixing a soybean feed material with wheat as disclosed in U.S. Pat. No. 6,090,416; and liquid Saccharopolyspora solubles. However, there is still a need for inexpensive and more efficient additives.

PGPUB-DOCUMENT-NUMBER: 20020127299

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020127299 A1

TITLE: FERULIC ACID DECARBOXYLASE

PUBLICATION-DATE: September 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
AGO, SHOJI	INASHIKI-GUN		JP	
KIKUCHI, YASUHIRO	TSUKUBA-SHI		JP	

APPL-NO: 09/ 335710

DATE FILED: June 18, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	25026/97	1997JP-25026/97	February 7, 1997

US-CL-CURRENT: 426/11

ABSTRACT:

The present invention relates to a protein having the amino acid sequence represented by SEQ ID NO: 1, or a protein having ferulic acid decarboxylase activity and having an amino acid sequence wherein one or more amino acid residues are deleted, substituted or added in the amino acid sequence represented by SEQ ID NO: 1; a gene encoding said protein; a recombinant vector comprising said gene; a transformant carrying said recombinant vector; a process for producing 4-vinylguaiacol, vanillin or vanillic acid, or a distilled liquor, wherein an enzyme source having ferulic acid decarboxylase activity which is derived from said transformant is used; and a process for producing a distilled liquor, wherein yeast having an enhanced ferulic acid decarboxylase activity is used.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (8):

[0007] It is known that distilled liquors having an excellent flavor can be produced by adding hydroxycinnamic acid ester hydrolase, or a koji mold having a high productivity of hydroxycinnamic acid ester hydrolase, (Japanese

Published Unexamined Patent Application No. 115957/95) or **ferulic acid esterase** [Nippon Nogeikagaku Kaishi, 70(6), 684-686 (1996)] to liberate ferulic acid into moromi.

PGPUB-DOCUMENT-NUMBER: 20010041203

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010041203 A1

TITLE: Method of removing off-flavor from foods and deodorizer

PUBLICATION-DATE: November 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Uno, Kazutaka	Tsuchiura-shi		JP	
Saitoh, Chiaki	Inashiki-gun		JP	
Egi, Makoto	Tokyo		JP	
Ago, Shoji	Kitakyushu-shi		JP	

APPL-NO: 09/ 826954

DATE FILED: April 6, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	2000-104277	2000JP-2000-104277	April 6, 2000

US-CL-CURRENT: 426/488, 422/5 , 424/76.1 , 426/237 , 426/541 , 426/61 , 426/648 , 426/649 , 426/650

ABSTRACT:

The present invention provides an effective method of removing off-flavor from foods such as seafood, meat products and vegetables, which comprises causing a polymer of phenol compounds having a styrene structure and/or a reaction mixture produced by conducting an oxidation reaction of the phenol compounds having a styrene structure in the presence of oxygen to be present in the food. Also provided is a deodorizer comprising a polymer of phenol compounds having a styrene structure.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (37):

[0034] Ferulic acid can be obtained by decomposing ferulic acid ester existing in fibrous substances such as arabinoxylan and pectin, which are one of the cell wall components of plants, in the presence of **ferulic acid esterase** in the usual way. Examples of plants known to contain ferulic acid or ferulic acid ester are cereals (e.g. rice, wheat, barley and buckwheat), potatoes (e.g. potatoes and sweet potatoes), fruits (e.g. grapes, apples and citrus fruits) and vegetables.

Summary of Invention Paragraph - BSTX (74):

[0071] As the first step, cereals such as rice (usually containing ferulic acid or ester thereof) as a raw material are liquefied and saccharified using a filamentous fungus such as rice koji. In this step, liquefying enzyme such as .alpha.-amylase and saccharifying enzyme such as glucoamylase may be added if necessary. Then, yeast is added to cause fermentation and the obtained fermentation liquor (moromi) is filtered to obtain a cooking liquor as the filtrate. The filamentous fungus used above has also fibrous hydrolase activity and **ferulic acid esterase** activity and contributes to decomposition of fibers in cereals and liberation of ferulic acid.

PGPUB-DOCUMENT-NUMBER: 20010014467

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010014467 A1

TITLE: Production of vanillin

PUBLICATION-DATE: August 16, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Narbad, Arjan	Norfolk		GB	
Rhodes, Michael John Charles	Norfolk		GB	
Gasson, Michael John	Norfolk		GB	
Walton, Nicholas John	Norfolk		GB	

APPL-NO: 09/ 733383

DATE FILED: December 7, 2000

RELATED-US-APPL-DATA:

child 09733383 A1 20001207

parent division-of 09155183 19990503 US PENDING

child 09155183 19990503 US

parent a-371-of-international PCT/GB97/00809 19970324 GB UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	9606187.4	1996GB-9606187.4	March 23, 1996

US-CL-CURRENT: 435/147, 435/189 , 435/252.34

ABSTRACT:

A method of producing vanillin comprising the steps of: (1) providing trans-ferulic acid or a salt thereof; and (2) providing trans-ferulate: CoASH ligase activity (enzyme activity I), trans-feruloyl ScoA hydratase activity (enzyme activity II), and 4-hydroxy-3-methoxyphenyl-.beta.-hydro- xy-propionyl SCoA (HMPHP SCoA) cleavage activity (enzyme activity III). Conveniently the enzymes are provided by Pseudomonas fluorescens Fe3 or a mutant or derivative thereof. Polypeptides with enzymes activities II and III and polynucleotides encoding said polypeptides. Use of said polypeptides or said polynucleotides in a method for producing vanillin.

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Summary of Invention Paragraph - BSTX (49):

[0049] Trans-ferulic acid or a salt thereof is readily available from plant material. Suitably, trans-ferulic acid or a salt thereof is released from the plant material by the action of **ferulic acid esterase**. Thus, in a particularly preferred embodiment of the invention the trans-ferulic acid or salt thereof is provided by the action of **ferulic acid esterase** on plant material.

Summary of Invention Paragraph - BSTX (50):

[0050] Trans-ferulic acid and trans4-coumaric acid can together represent up to 1.5% by weight of the cell walls of temperate grasses (R. D. Hartley and E. C. Jones, Phytochemistry 16, 1531-1534 (1977)). Trans-ferulic acid is reported to comprise 0.5% (w/w) of wheat bran (M. C. Ralet, J.-F. Thibault and G. Della Valle, J. Cereal Sci. 11, 249-259 (1990)), 3.1% of maize bran (L. Saulnier, C. Marot, E. Chanliaud and J.-F. Thibault, Carbohydr. Polym. 26, 279-287 (1995)) and 0.8% of sugar beet pulp (V. Micard, G. M. G. C. Renard and J.-F. Thibault, Lebensm.-Wiss. u-Technol. 27, 59-66 (1994)). These materials are amongst the preferred sources of trans-ferulic acid. Since trans-ferulic acid is present esterified with cell-wall polysaccharides, hydrolysis is essential. Alkaline or acid hydrolysis is possible, but enzymic hydrolysis is preferred. Typically, the initial step is the partial enzymic hydrolysis of carbohydrates (arabinans, xylans, rhamnogalacturanans) to which trans-ferulate is linked, followed by the release of trans-ferulate from the oligosaccharide fragments by trans-**ferulic acid esterase** activity. In practice, both steps may occur simultaneously in the reaction mixture. Descriptions of representative laboratory-scale processes are available in the literature (for example see L. P. Christov and B. A. Prior, Enzyme Microb. Technol. 15, 460-475 (1993)); C. B. Faulds and G. Williamson, Appl. Microbiol. Biotechnol. 43, 1082-1087 (1995); C. B. Faulds, P. A. Kroon, L. Saulnier, J.-F. Thibault and G. Williamson, Carbohydrate Polymers 27, 187-190 (1995)). Phenolic acid-releasing enzymes have been reported from a number of microorganisms, including Streptomyces olivochromogenes (C. B. Faulds and G. Williamson, J. Gen. Microbiol. 137, 2337-2345 (1991)), Penicillium pinophilum (A. Castanares, S. I. McCrae and T. M. Wood, Enzyme Microb. Technol. 14, 875-884 (1992)), Neocallimastix spp. (W. S. Borneman, R. D. Hartley, W. H. Morrison, D. E. Akin and L. G. Ljungdahl, Appl. Microbiol. Biotechnol. 33, 345-35,1 (1990)), Schizophyllum commune (R. C. MacKenzie and D. Bilous, Appl. Envir. Microbiol. 54, 1170-1173 (1988)) and Aspergillus spp. (M. Tenkanen, J. Schuseil, J. Puls and K. Poutanen, J. Biotechnol. 18, 69-84 (1991); C. B. Faulds and G. Williamson, Microbiology 140, 779-787 (1994)). A trans-**ferulic acid esterase** (XYLD) has been characterised from Pseudomonas fluorescens subsp. cellulosa, together with an arabinofuranosidase (XYLC) and an endoxylanase (XYLB; L. M. A. Ferreira, T. M. Wood, G. Williamson, C. B. Faulds, G. P. Hazlewood and H. J. Gilbert, Biochem. J. 294, 349-355 (1993)). The genes for all three enzymes have been isolated (G. P. Hazlewood and H. J. Gilbert, in "Xylans and Xylanases", eds. J. Visser, G. Beldman, M. A. Kusters-van Someren and A. G. J. Voragen, Elsevier, Amsterdam, pp 259-273 (1992)). All of these references are incorporated herein by reference.

Summary of Invention Paragraph - BSTX (51):

[0051] Thus, advantageously the trans-ferulic acid or a salt thereof may be provided by the action of trans-**ferulic acid esterase** on said ester. More particularly, it is advantageous to introduce a gene encoding said esterase into a host cell or organism which is being used in the methods of the invention. Thus, it is convenient to introduce a trans-**ferulic acid esterase** gene, such as the aforementioned XYLD gene, into a plant which is being used in the methods of the invention.

Claims Text - CLTX (11):

11. A method according to any one of claims 1 to 10 wherein the trans-ferulic acid or salt thereof is provided by action of trans-**ferulic acid esterase** on plant material, said plant material containing an ester of trans-ferulic acid.

US-PAT-NO: 6534286

DOCUMENT-IDENTIFIER: US 6534286 B1

TITLE: Protein production in Aureobasidium pullulans

DATE-ISSUED: March 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Li; Xin-Liang	Athens	GA	N/A	N/A
Ljungdahl; Lars G.	Athens	GA	N/A	N/A

APPL-NO: 09/ 595344

DATE FILED: June 15, 2000

US-CL-CURRENT: 435/69.1, 435/183 , 435/200 , 435/252.1 , 435/252.3 , 435/254.1 , 435/254.11 , 435/254.2 , 435/320.1 , 435/325 , 435/69.8 , 435/69.9 , 536/23.1 , 536/23.2 , 536/24.1

ABSTRACT:

The present disclosure provides methods and DNA molecules for the synthesis of heterologous proteins in the fungus Aureobasidium pullulans either intracellularly or with secretion out of the cells using a regulated xylanase promoter and for secreted protein synthesis, a signal sequence. Further described are kits containing host cells for recombinant protein production, a vector containing an XynA transcription regulatory sequence, and instructions for using the vector to transform the host cells.

34 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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Brief Summary Text - BSTX (8):

Hemicellulose, second only to cellulose in abundance on earth, comprises xylan as the main constituent. Xylan is a hetero-polymer comprising beta-1,4-linked xylose units as a backbone and side chains which contain pentose, hexose, and acetyl groups. The pentose (arabinose) is esterified to free and lignin phenolic (feruloyl and p-coumaroyl) groups [Christov and Prior (1993) Enzyme Microb. Technol. 15:460-475]. Xylan can be readily converted to xylose and other monomeric sugars through either chemical or enzymatic

hydrolysis of agricultural and forestry waste biomass. Enzymatic degradation of hemicelluloses requires the participation of several enzymes including xylanase (EC3.2.1.8), .beta.-xylosidase (EC3.2.1.37), .alpha.-L-arabinofuranosidase (EC3.2.1.55), .alpha.-glucuronidase (EC3.2.1.1), acetyl xylan esterase (EC3.1.1.6) as well as p-**coumaroyl and feruloyl esterases** [Borneman et al. (1993) In: Hemicellulose and Hemicellulases, M. P. Coughlan and G. Hazlewood (ed.), Portland Press, Cambridge, UK, pp. 85-102; Christov and Prior (1993) supra]. Due to efficient conversion of agricultural plant residues to xylose and widespread use of xylitol, an alcohol derived from xylose in food products, using xylose as an inducer in large-scale fermentation has become cost effective in comparison to other commonly used inducers for fermentation.

US-PAT-NO: 6534101

DOCUMENT-IDENTIFIER: US 6534101 B1

TITLE: Enzymes mixture obtained from Penicillium funiculosum

DATE-ISSUED: March 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sabatier; Alain	Paris	N/A	N/A	FR
Fish; Neville Marshall	Bramhall	N/A	N/A	GB
Haigh; Nigel Paterson	Huddersfield	N/A	N/A	GB

APPL-NO: 09/ 462246

DATE FILED: April 7, 2000

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	98401101	September 19, 2001

PCT-DATA:
APPL-NO: PCT/IB99/00856
DATE-FILED: May 6, 1999
PUB-NO: WO99/57325
PUB-DATE: Nov 11, 1999
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 426/53, 426/18 , 426/44 , 426/656 , 435/183 , 435/197 , 435/203 , 435/209 , 435/254.5 , 435/256.3

ABSTRACT:

The present invention relates to a novel micro-organism, Penicillium funiculosum, to a new enzymes mixture obtained from it, and nucleic acid sequences encoding such enzymes.

18 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Brief Summary Text - BSTX (47):

We obtain a new enzymes mixture produced by Penicillium funiculosum. This enzymes mixture contains new enzymes such as cellulases, .beta.-glucanases, xylanases, xylanase accessory enzymes such as arabinofuranosidase and **feruloyl esterases**.

Brief Summary Text - BSTX (49):

The enzyme preparation is characterised by assays that include assays for cellulase, cellobiohydrolase, .beta.-glucosidase, endo-1,3(4)-.beta.-glucanase, laminarinase endo-1,4-.beta.-xylanase (using different substrates), .beta.-xylosidase, arabinofuranosidase and **feruloyl esterase** (using different substrates) activities.

Brief Summary Text - BSTX (103):

1.12 **Feruloyl Esterase** by the FAXX Method

Brief Summary Text - BSTX (104):

An assay of **feruloyl esterase (ferulic acid esterase)** is based on the enzymatic hydrolysis of O-[5-O-(trans-feruloyl)-.alpha.-L-arabinofuranosyl]-(1.fwdarw.3)-O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-D-xyfopyranose (FAXX). FAXX is prepared from enzyme-hydrolysed wheat bran, purified and characterised by NMR. FAXX hydrolysis is measured spectophotometrically.

Brief Summary Text - BSTX (106):

One unit of **feruloyl esterase** activity on FAXX is defined as the amount of enzyme which converts 1 .mu.mole substrate to product per minute under the conditions of the assay (37.degree. C. and pH 6.0).

Brief Summary Text - BSTX (107):

1.13 **Feruloyl Esterase** by the Ara.sub.2 F Method

Brief Summary Text - BSTX (108):

An assay of **feruloyl esterase (ferulic acid esterase)** is based on the enzymatic hydrolysis of Ara.sub.2 F (ferulic acid linked 1,2 to arabinose). Ara.sub.2 F is prepared from enzyme-hydrolysed sugar beet pulp, purified and characterised by NMR. Ara.sub.2 F hydrolysis is measured spectophotometrically.

Brief Summary Text - BSTX (110):

One unit of **feruloyl esterase** activity on Ara.sub.2 F is defined as the amount of enzyme which converts 1 .mu.mole substrate to product per minute under the conditions of the assay (37.degree. C. and pH 6.0).

Brief Summary Text - BSTX (111):

1.14 **Feruloyl Esterase** by the Hydrolysis of Methyl Esters: Methyl Ferulic Acid (MFA); Methyl Caffeic Acid (MCA); Methyl Sinapic Acid (MSA); Methyl p-Coumaric Acid (MPCA) Methods

Brief Summary Text - BSTX (112):

An assay of **feruloyl esterase (ferulic acid esterase)** is based on the enzymatic hydrolysis of methyl esters of ferulic acid (MFA), caffeic acid (MCA), sinapic acid (MSA) and p-coumaric acid (MpCA). Methyl ester hydrolysis is measured in 0.1M MOPS buffer, pH 6.0 at 37.degree. C. Assays are based on two different techniques.

Brief Summary Text - BSTX (115):

One unit of **feruloyl esterase** activity is defined as the amount of enzyme which converts 1 .mu.mole substrate to product per minute under the conditions of the assay (37.degree. C. and pH 6.0).

Brief Summary Text - BSTX (186):

3.3 Properties of **Feruloyl Esterases**

Brief Summary Text - BSTX (189):

The enzymes mixture contains at least two distinct **feruloyl esterases**. One of these (FaeB) has a molecular weight of 38,945-41,051 Da by mass spectrometry (35.450 Da from the primary amino acid sequence and 37 kDa by SDS-PAGE). FaeB has a pI of 4.2, it is a type B **feruloyl esterase** and is specific for MpCA and Ara.sub.2 F substrates (activity against MpCA, MCA, MFA and Ara.sub.2 F; but not against MSA and FAXX).

Brief Summary Text - BSTX (190):

The other **feruloyl esterase** (FaeA) has a molecular weight of 29 kDa (by SDS-PAGE). FaeA has a pI of 4.65, it is a type A **feruloyl esterase** and is specific for FAXX and MSA substrates (activity against MSA, MCA, MFA and FAXX but not MpCA Ara.sub.2 F).

Brief Summary Text - BSTX (193):

The stained IEF gel indicates the presence of very many proteins in cellulase with pI's ranging from very acidic (pI 2.4) to about pI 7. Most of the proteins are acidic (pI range 2.4-5). Two peaks of **feruloyl esterase** activity were detected in fractions cut from the gel. One, corresponding to FaeB, had a pI of 4.2 and activity only against MFA and MpCA (not MSA). The other, corresponding to FaeA, had a pI of 4.65 and activity against all three substrates tested .

Brief Summary Text - BSTX (196):

The enzymes mixture contains at least two distinct **feruloyl esterases**. One corresponding to FaeB (pI 4.2) has a molecular weight of 37 kDa. The other, corresponding to FaeA (pI 4.65) has a molecular weight of 29 kDa.

Brief Summary Text - BSTX (199):

Assays for **feruloyl esterase** activity performed on the enzymes mixture using the spectrophotometric method

Brief Summary Text - BSTX (200):

The enzymes mixture contains activity against all the substrates tested. With the methyl esters, activity is highest against MpCA and lowest against MSA. The activities against Ara.sub.2 F and FAXX are higher than against the methyl esters which is indicative that the esterase activities are due to true **feruloyl esterases** and not general esterases or side activities of other cell wall-degrading esterases (e.g. acetyl xylan esterase, pectin esterase).

Brief Summary Paragraph Table - BSTL (9):

TABLE A Relative activities against relevant different substrates Results with Penicillium Methods used in the tests funiculosum Cellulase (DNS CMC method, pH 5.0) [1.1] 3.14 Cellobiohydrolase (p-nitrophenyl .beta.-D-cellobiopyranoside 0.022 method, pH 5.0) [1.2] .beta.-Glucosidase (p-nitrophenyl .beta.-D-glucobiopyranoside 0.157 method, pH 5.0) [1.3] Endo-1,3(4)-.beta.-glucanase (DNS barley .beta.-glucan method, pH 7.23 5.0) [1.4] Endo-1,3(4)-.beta.-glucanase (azo-barley .beta.-glucan method, pH 1+/- 4.6) [1.5] Laminarinase (DNS laminarin method, pH 5.0) [1.6] 0.30 Endo-1,4-.beta.-xylanase (DNS birchwood xylan method, pH 9.16 3.5) [1.7] Endo-1,4-.beta.-xylanase (DNS wheat arabinoxylan method, pH 8.67 3.5) [1.8] Endo 1,4-.beta. xylanase (viscometric wheat arabinoxylan 9.80 method, pH 5.5) [1.9] .beta.-Xylosidase (p-nitrophenyl .beta.-D-xylobiopyranoside 0.0047 method) [1.10] .alpha.-N-Arabinofuranosidase (p-nitrophenyl .alpha.-L-arabinofura- 0.0017 noside method) [1.11] **Feruloyl esterase** (FAXX method) [1.12] 0.000254 **Feruloyl esterase** (Ara.sub.2 F method) [1.13] 0.000349 **Feruloyl esterase** (MFA spectrophotometric method) [1.14] 0.000135 **Feruloyl esterase** (MCA spectrophotometric method) [1.14] 0.000174 **Feruloyl esterase**

(MSA spectrophotometric method) [1.14] 0.000049 **Feruloyl esterase** (MpCA spectrophotometric method) 0.000216 [1.14]

Claims Text - CLTX (3):

3. The supernatant of claim 1 comprising xylanases, .beta.-glucanases, cellulases and **feruloyl esterases**.

Claims Text - CLTX (15):

15. The enzyme mixture of claim 13, comprising xylanases, .beta.-glucanases, cellulases and **feruloyl esterases**.

Claims Text - CLTX (17):

17. An enzyme mixture comprising xylanases, .beta.-glucanases, cellulases and **feruloyl esterases,** wherein optimal activity of the xylanases is achieved at a pH between pH 3.0 and 5.0, and wherein the enzyme mixture improves feed digestibility when added to feed as compared to the feed not having the enzyme mixture.

Other Reference Publication - OREF (2):

Ralet et al., "Degradation of feruloylated oligosaccharides from sugar-beet pulp and wheat ran by **ferulic acid esterases** from Aspergillus niger", Carbohyrate Research 263 (1994), pp. 257-269.

US-PAT-NO: 6468955

DOCUMENT-IDENTIFIER: US 6468955 B1

TITLE: Laundry detergent and/or fabric care compositions comprising a modified enzyme

DATE-ISSUED: October 22, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Smets; Johan	Lubbeek	N/A	N/A	BE
Bettiol; Jean-Luc Philippe	Brussels	N/A	N/A	BE
Boyer; Stanton Lane	Fairfield	OH	N/A	N/A
Busch; Alfred	Londerzeel	N/A	N/A	BE

APPL-NO: 09/ 674478

DATE FILED: November 1, 2000

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
WO	PCT/US98/08856	May 1, 1998

PCT-DATA:
APPL-NO: PCT/US99/09453
DATE-FILED: April 30, 1999
PUB-NO: WO99/57252
PUB-DATE: Nov 11, 1999
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 510/392, 435/174 , 510/276 , 510/305 , 510/320 , 510/374

ABSTRACT:

Modified enzymes which comprise a catalytically active amino acid sequence of an enzyme, linked via a non-mino acid linking region to an amino acid sequence comprising a Cellulose Binding Domain. The present invention further relates to laundry detergent and/or fabric care compositions comprising such modified enzymes. These compositions provide a higher effective concentration of the enzyme at its substrate location and therefore, improved enzymatic benefits.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (36):

Also suitable are xylan degrading enzymes. By xylan degrading enzyme it is meant herein any enzyme which degrade, for instance hydrolyse and/or modify, xylan containing polymers which are associated with hemicellulose and other plant polysaccharides. The xylan degrading alkaline enzyme can be a single xylan degrading activity species or a mixture of the iso-enzymes obtained via the purification of the crude xylan degrading alkaline enzyme mixure. The xylan degrading enzymes of interest are the endo- and exo-Xylanases hydrolysing Xylan in endo- or in exo fashion: endo-1,3 beta Xylosidase (E.C. 3.2.1.32), the endo-1,4-beta Xylanase (E.C. 3.2.1.8), 1,3-beta D Xylans Xylohydrolase, (E.C. 3.2.1.72), 1,4-beta D Xylans Xylohydrolase, (E.C. 3.2.1.37). Other Xylan degrading alkaline enzymes of interest remove substitutions from the main xylan polymer such as Acetylxylan esterase; Glucuronoarabinoxylan endo-1,4-xylanase (E.C. 3.2.1.136), arabinosidase (E.C.3.2.1.55) and **ferulic esterase and coumaric acid esterase**. These enzymes remove respectively the acetylation, 4-O-methyl glucuronic side chains; the L-arabinose side chains and ferulic acid cross linkages and p-coumaric side chains from the main xylan polymer.

US-PAT-NO: 6468566

DOCUMENT-IDENTIFIER: US 6468566 B2

TITLE: Ferulic acid decarboxylase

DATE-ISSUED: October 22, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ago; Shoji	Ami-machi	N/A	N/A	JP
Kikuchi; Yasuhiro	Tsukuba	N/A	N/A	JP

APPL-NO: 09/ 335710

DATE FILED: June 18, 1999

PARENT-CASE:

This application is a division of application Ser. No. 09/018,787 filed Feb. 4, 1998, now U.S. Pat. No. 5,955,137.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	9-25026	February 7, 1997

US-CL-CURRENT: 426/52, 426/11 , 426/29 , 426/49 , 426/592 , 426/7

ABSTRACT:

The present invention relates to a protein having the amino acid sequence represented by SEQ ID NO: 1, or a protein having ferulic acid decarboxylase activity and having an amino acid sequence wherein one or more amino acid residues are deleted, substituted or added in the amino acid sequence represented by SEQ ID NO: 1; a gene encoding said protein; a recombinant vector comprising said gene; a transformant carrying said recombinant vector; a process for producing 4-vinylguaiacol, vanillin or vanillic acid, or a distilled liquor, wherein an enzyme source having ferulic acid decarboxylase activity which is derived from said transformant is used; and a process for producing a distilled liquor, wherein yeast having an enhanced ferulic acid decarboxylase activity is used.

2 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Brief Summary Text - BSTX (8):

It is known that distilled liquors having an excellent flavor can be produced by adding hydroxycinnamic acid ester hydrolase, or a koji mold having a high productivity of hydroxycinnamic acid ester hydrolase, (Japanese Published Unexamined Patent Application No. 115957/95) or **ferulic acid esterase** [Nippon Nogeikagaku Kaishi, 70(6), 684-686 (1996)] to liberate ferulic acid into moromi.

US-PAT-NO: 6368833

DOCUMENT-IDENTIFIER: US 6368833 B1

TITLE: Esterases, DNA encoding therefor and vectors and host incorporating same

DATE-ISSUED: April 9, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Borneman; William S.	San Carlos	CA	N/A	N/A
Bower; Benjamin S.	Pacifica	CA	N/A	N/A

APPL-NO: 08/ 952445

DATE FILED: November 18, 1997

PARENT-CASE:

This application is a continuation of application Ser. No. 08/722,713 filed Sep. 30, 1996, now abandoned.

PCT-DATA:
APPL-NO: PCT/US97/17614
DATE-FILED: September 29, 1997
PUB-NO: WO98/14594
PUB-DATE: Apr 9, 1998
371-DATE: Nov 18, 1997
102(E)-DATE:Nov 18, 1997

US-CL-CURRENT: 435/91.1, 435/197 , 435/252.3 , 435/320.1 , 435/325 , 536/23.1 , 536/23.2

ABSTRACT:

A novel DNA is provided which encodes an enzyme having esterolytic activity isolated from Aspergillus. Also provided for is a method of isolating DNA encoding an enzyme having esterolytic activity from organisms which possess such DNA, transformation of the DNA into a suitable host organism, expression of the transformed DNA and the use of the expressed esterase protein in feed as a supplement, in textiles for the finishing of such textiles prior to sale, in starch processing or production of foods such as baked bread.

17 Claims, 9 Drawing figures

Exemplary Claim Number: 9

Number of Drawing Sheets: 12

---------- KWIC ---------

Brief Summary Text - BSTX (4):

Enzyme hydrolysis of xylan to its monomers requires the participation of several enzymes with different functions. These are classified in two groups based on the nature of the linkages that they cleave. The first group of enzymes is hydrolases (EC 3.2.1) involved in the hydrolysis of the glycosidic bonds of xylan. These include endo-xylanases (EC 3.2.1.8) which randomly dismember the xylan backbone into shorter xylooligosaccharides; .beta.-xylosidase (EC 3.2.1.37) which cleave the xylooligosaccharides in an exo-manner producing xylose; .alpha.-L-arabinofuranosidase (EC 3.2.1.55); and .alpha.-glucoronidase (EC 3.2.1.1) which remove the arabinose and 4-O-methylglucuronic acid substituents, respectively, from the xylan backbone. The second group includes enzymes that hydrolyze the ester linkages (esterase, EC 3.1.1) between xylose units of the xylan polymer and acetyl groups (acetyl xylan esterase, EC 3.1.1.6) or between arabinosyl groups and phenolic moieties such as ferulic acid (**feruloyl esterase) and p-coumaric** acid (**coumaroyl esterase**).

Brief Summary Text - BSTX (5):

Faulds et al., reported two forms of **ferulic acid esterase** isolated from Aspergillus niger. The different esterases were distinguished on the basis of molecular weight and substrate specificity (Faulds et al., Biotech. Appl. Biochem., vol. 17, pp. 349-359 (1993)). Brezillon et al. disclosed the existence of at least two **cinnamoyl esterases** which were believed to be distinct from the **ferulic acid esterases** shown in the prior art (Brezillon et al., Appl. Microb. Biotechnol., vol. 45, pp. 371-376 (1996)). A **ferulic acid esterase** called FAE-III was isolated from Aspergillus niger CBS 120.49 and shown to act together with xylanase to eliminate nearly all of the ferulic acid and low molecular mass xylooligosaccharides in a wheat bran preparation; ferulic acid was also removed without the addition of xylanase, albeit at a lower level. Faulds et al. further isolated and partially characterized FAE-III from Aspergillus niger CBS 120.49 grown on oat spelt xylan (Faulds et al., Microbiology, vol. 140, pp. 779-787 (1994)) and showed it to have a pI of 3.3, a molecular weight of 36 kD (SDS-PAGE) and 14.5 kD (Gel Filtration method), a pH optimum of 5 and a temperature optimum of 55-60.degree. C.; microcrystalline cellulose binding was also detected. The authors theorized that FAE-II may be a proteolytically modified FAE-III. Recently, the various known **ferulic acid esterases** derived from Aspergillus niger have been distinguished based on their distinct substrate specificity and it was noted that FAE-II and FAE-III were unable to release ferulic acid from sugar beet pulp (Brezillon et al., supra).

Detailed Description Text - DETX (2):

"Esterase" or "esterolytic activity" means a protein or peptide which exhibits esterolytic activity, for example, those enzymes having catalytic

activity as defined in enzyme classification EC 3.1.1. Esterolytic activity may be shown by the ability of an enzyme or peptide to cleave ester linkages, for example, feruloyl, coumaroyl or acetyl xylan groups, from organic compounds in which they are known to exist, e.g., primary and secondary cell walls. Preferably, the esterase comprises an esterolytic activity which cleaves the ester linkage of phenolic esters such as:
[5O-((E)-feruloyl)-.alpha.-L-arabinofuranosyl](1.fwdarw.3)-O-.beta.-D-xylo pyranosyl-(1.fwdarw.4)-D-xylopyranose (also known as FAXX);
[5-O-((E)-feruloyl)-.alpha.-L-arabinofuranosyl](1.fwdarw.3)-O-.beta.-D-xyl opyranose (also known as FAX);
O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-O-[5-O-((E)-feruloyl)-.alpha.-arabin ofuranosyl-(1.fwdarw.3)]-O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-D-xylopyrano se (also known as FAXXX);
[5-O-((E)-p-coumaroyl)-.alpha.-L-arabinofuranosyl](1.fwdarw.3)-O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-D-xylopyranose (also known as PAXX);
[5-O-((E)-p-coumaroyl)-.alpha.-L-arabinofuranosyl](1.fwdarw.3)-O-.beta.-D-xylopyranose (also known as PAX);
O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-O-[5-O-((E)-p-coumaroyl)-.alpha.-ara binofuranosyl-(1.fwdarw.3)]-O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-D-xylopyr anose (also known as PAXXX) and other ester linked phenolic oligosaccharides as are known in the art. Such **esterases are generally referred to as ferulic acid esterase** (FAE) or enzymes having **feruloyl esterase** activity. It has surprisingly been discovered that an **esterase having ferulic acid esterase** activity which may be purified from Aspergillus niger, as described herein, and having an amino acid sequence as shown in FIG. 4, further has activity on sugar beet pulp and also proteolytic and lipolytic activity. Thus, according to a particularly preferred embodiment of the present invention, an esterase and/or a DNA encoding that esterase is provided which esterase also has lipolytic and/or proteolytic activity. Accordingly, the esterase of the invention having measurably significant esterolytic activity on feruloyl and coumaroyl esters also has proteolytic and lipolytic activity.

Detailed Description Text - DETX (17):

Purification and Isolation of Peptides Comprising **Ferulic Acid Esterase** Activity and Design of Degenerate DNA Fragments for PCR

Detailed Description Paragraph Table - DETL (5):

TABLE 3 Release of ferulic acid from sugar beet pulp with **ferulic acid esterase** Ferulic acid released from sugar beet pulp Enzyme 12 hrs 24 hrs treatment .mu.g % .mu.g % FAE 15.3 2.7 26.2 4.6 Xylanase 0.5 0.1 0.6 0.1 FAE + 27.1 4.8 49.7 8.7 Xylanase Buffer Control 0.2 0.04 0.2 0.04 Inactivated 0.2 0.03 0.2 0.04 FAE

Other Reference Publication - OREF (1):

Bartolome, et al., "Influence of different xylanases on activity of **ferulic acid esterase** on wheat bran," Biotechnol. Appl. Biochem., V. 22, pp. 65-73, 1995.

Other Reference Publication - OREF (2):

Borneman, et al., "Assay for trans-p-**Coumaroyl Esterase** Using a Specific Substrate from Plant Cell Walls," Analytical Biochemistry, V. 190, pp. 129-133 1990.

Other Reference Publication - OREF (4):

Borneman, et al., "Purification and Partial Characterization of Two **Feruloyl Esterases** from the Anaerobic Fungus Neocallimastix Strain MC-2," Applied and Environmental Microbiology, V. 58 (11), pp. 3762-3766, Nov. 1992.

Other Reference Publication - OREF (5):

Brezillon, et al., "Novel **ferulic acid esterases** are induced by growth of Aspergillus niger on sugar-beet pulp, " V. 45, pp. 371-376, 1996.

Other Reference Publication - OREF (6):

Castanares, et al., "Purification and properties of a feruloyl/p-**coumaroyl esterase** from the fungus Penicillium pinophilum," Enzyme Microb. Technbol., V. 14, pp. 875-884, Nov. 1992.

Other Reference Publication - OREF (11):

Dugelay, et al., "Role of **Cinnamoyl Esterase** Activities from Enzyme Preparations on the Formation of Volatile Phenols during Winemaking," J. Agric. Food Chem., V. 41, pp. 2092-2096, 1993.

Other Reference Publication - OREF (13):

Faulds, et al., "A major bioactive component of plant cell walls, ferulic acid, influences **feruloyl esterase** production in Aspergillus niger," Biochemical Society Transactions, V. 24, pp. 386S, 1996.

Other Reference Publication - OREF (14):

Faulds, et al., "**Ferulic acid esterase** from Aspergillus niger. purification and partial characterization of two forms from a commercial source of pectinase," Biotechnol. Appl. Beochem., V. 17, pp. 349-359, 1993.

Other Reference Publication - OREF (16):

Faulds, et al., "Release of ferulic acid from plant polysaccharides by **ferulic acid esterase** from Streptomyces olivochromogenes," Carbohydrate Polymers, V. 21 pp. 153-155, 1993.

Other Reference Publication - OREF (17):

Faulds, et al., "Release of ferulic acid from wheat bran by a **ferulic acid esterase** (FAE-III) from Aspergillus niger," Appl. Microbiol. Biotechnol, V. 43, pp. 1082-1087, 1995.

Other Reference Publication - OREF (19):

Faulds, et al., "The purification and characterization of 4-hydroxy-3-methoxycinnamic (**ferulic) acid esterase** from Streptomyces olivochromogenes," Journal of General Microbiology, V. 137, pp. 2339-2345, 1991.

Other Reference Publication - OREF (21):

Hatfield, et al., "Synthesis of Methyl 5-O-trans-Feruloyl-.alpha.-L-arabinofuranoside and Its Use as a Substrate to Assess **Feruloyl Esterase** Activity," Analytical Biochemistry, V. 194, pp. 25-33, 1991.

Other Reference Publication - OREF (25):

MacKenzie, et al., "**Ferulic Acid Esterase** Activity from Schizophyllum commune," Applied and Environmental Microbiology, V. 54 (5), pp. 1170-1173, May 1988.

Other Reference Publication - OREF (26):

McCallum, et al., "Spectrophotometric Assay and Electrophoretic Detection of trans-**Feruloyl Esterase** Activity," Analytical Biochemistry, V. 196, pp. 360-366 1991.

Other Reference Publication - OREF (27):

McCrae, et al., "Xylan-degrading enzyme system produced by the fungus Aspergillus awamori: isolation and characterization of a **feruloyl esterase and a p-coumaroyl esterase,**" Enzyme Microb. Technol., V. 16, pp. 826-834, Oct. 1994.

Other Reference Publication - OREF (29):

Tenkanen, et al., "Production, purification and characterization of an **esterase liberating phenolic** acids from lignocellulosics," Journal of Biotechnology, V. 18, pp. 69-84, 1991.

US-PAT-NO: 6365390

DOCUMENT-IDENTIFIER: US 6365390 B1
See image for Certificate of Correction

TITLE: **Phenolic acid esterases,** coding sequences and methods

DATE-ISSUED: April 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Blum; David L.	San Diego	CA	N/A	N/A
Kataeva; Irina	Athens	GA	N/A	N/A
Li; Xin-Liang	Athens	GA	N/A	N/A
Ljungdahl; Lars G.	Athens	GA	N/A	N/A

APPL-NO: 09/ 390234

DATE FILED: September 3, 1999

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from United States Provisional Application No. 60/099,136, filed Sep. 4, 1998.

US-CL-CURRENT: 435/197, 435/183 , 435/252.3 , 435/320.1 , 530/350 , 536/23.1 , 536/23.2

ABSTRACT:

Described herein are four **phenolic acid esterases,** three of which correspond to domains of previously unknown function within bacterial xylanases, from XynY and XynZ of Clostridium thermocellum and from a xylanase of Ruminococcus. The fourth specifically exemplified xylanase is a protein encoded within the genome of Orpinomyces PC-2. The amino acids of these polypeptides and nucleotide sequences encoding them are provided. Recombinant host cells, expression vectors and methods for the recombinant production of **phenolic acid esterases** are also provided.

26 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

---------- KWIC ---------

Abstract Text - ABTX (1):

Described herein are four **phenolic acid esterases,** three of which correspond to domains of previously unknown function within bacterial xylanases, from XynY and XynZ of Clostridium thermocellum and from a xylanase of Ruminococcus. The fourth specifically exemplified xylanase is a protein encoded within the genome of Orpinomyces PC-2. The amino acids of these polypeptides and nucleotide sequences encoding them are provided. Recombinant host cells, expression vectors and methods for the recombinant production of **phenolic acid esterases** are also provided.

TITLE - TI (1):

Phenolic acid esterases, coding sequences and methods

Brief Summary Text - BSTX (2):

The field of the present invention is the area of enzymes which degrade plant cell walls, and certain other substrates, in particular, the **phenolic acid esterases, feruloyl esterases** and/or **coumaroyl esterase,** nucleotide sequences encoding them and recombinant host cells and methods for producing them.

Brief Summary Text - BSTX (3):

Plant cell wall material is one of the largest sources of renewable energy on earth. Plant cell walls are composed mainly of cellulose, hemicelluloses, lignin and pectin. Arabinoxylan is one of the main constituents of hemicelluloses. It is composed of a chain of .beta.(1.fwdarw.4) linked xylose units that are substituted by arabinose, acetate, and glucuronic acid. The arabinose has ester linked ferulic and p-coumaric acids [Borneman et al. (1993) In: Hemicellulose and Hemicellulases, Coughlan and Hazlewood, Eds., pp. 85-102]. Ferulic acid has been shown to link hemicellulose and lignin [Ralph et al. (1995) Carbohydrate Research 275:167-178]. **Feruloyl esterases** are involved in breaking the bond between the arabinose and ferulic acid, thus releasing the covalently bound lignin from hemicelluloses. **Feruloyl esterases** have been found in many bacteria as well as fungi, but have not been extensively studied nor is there much sequence data available [Christov and Prior (1993) Enzyme. Microb. Technol. 15(6):460-75].

Brief Summary Text - BSTX (4):

Clostridium thermocellum is a gram-positive bacterium that produces a multienzymatic structure termed the cellulosome. The cellulosome is one of the most active cellulose degrading complexes described to date. The cellulosome has a multi-polypeptide structure, including a scaffolding subunit which has nine cohesins binding to nine catalytic subunits, a dockerin domain for attachment to the cell wall, and a cellulose binding domain [Felix and

Ljungdahl (1993) Annu. Rev. Microbiol. 47:791-819]. The catalytic subunits include endoglucanase, cellobiohydrolase, lichenase, and xylanase, many of which have been cloned and sequenced. They all have multidomain structures that include at least a dockerin domain for binding to the scaffolding domain, a linker, and a catalytic domain. They may also contain cellulose binding domains and fibronectin-like domains. There are reports that some enzymatic components may have more than one catalytic domain. Two of these are xylanase Y [XynY, Fontes et al. (1995) Biochem. J. 307: 151-158] and xylanase Z [XynZ, Grepinet et al. (1988) J. Bacteriol. 170(10):4582-8]. XynY has a C-terminal domain whereas XynZ N-terminal domain without any functions determined. Although enzymes with dual catalytic domains (xylanase and .beta.-glucanase) have been found in other bacteria [Flint et al. (1993) J. Bacteriol. 175:2943-2951] neither **phenolic acid esterase** nor bifunctional enzymes have been found in C thermocellum.

Brief Summary Text - BSTX (5):

There is a need in the art for **phenolic acid esterases, feruloyl esterases** and/or **coumaroyl esterases** in pure form which degrade plant cell wall materials, and certain other substrates, and for DNA encoding these enzymes to enable methods of producing ferulic acid and/or coumaric acid as well as facilitating degradation of plant cell wall materials.

Brief Summary Text - BSTX (7):

The present invention provides novel **phenolic acid esterases, having feruloyl esterase and coumaroyl esterase** activities, and coding sequences for same.

Brief Summary Text - BSTX (8):

One **phenolic acid esterase** of the present invention corresponds to a domain of previously unknown function from xylanase Y of Clostridium thermocellum. The recombinantly expressed domain polypeptide is active and has an amino acid sequence as given in FIG. 1 as "XynY_Clotm." The nucleotide sequence encoding the esterase polypeptide is given in Table 5, nucleotides 2383-3219, exclusive of translation start and stop signals. See also SEQ ID NOs:11 and 12.

Brief Summary Text - BSTX (9):

A second **phenolic acid esterase** of the present invention corresponds to a domain of previously unknown function of xylanase Z from C. thermocellum. The amino acid sequence of the esterase domain, which also is active when expressed as a recombinant polypeptide, is given in FIG. 1 as "XynZ_Clotm." The nucleotide sequence encoding this polypeptide is given in Table 6, nucleotides 58-858. The present invention further provides a **phenolic acid esterase** polypeptide further comprising a cellulose binding domain. A specifically identified cellulose binding domain has an amino acid sequence as given in Table 6, 289-400, with a corresponding coding sequence as given in Table 6,

nucleotides 867-1200. See also SEQ ID NOs:13 and 14.

Brief Summary Text - BSTX (10):

An additional object of the present invention is a **phenolic acid esterase (i.e., a feruloyl esterase)** derived from a previously uncharacterized portion of a Ruminococcus xylanase (See FIG. 1). The coding (nucleotides 2164-2895, exclusive of translation start and stop signals) and deduced amino acid sequences (amino acids 546-789) are given in Table 10. See also SEQ ID NOs: 15 and 16.

Brief Summary Text - BSTX (11):

The present invention further provides a feruloyl (**phenolic acid) esterase** from the anaerobic fungus Orpinomyces PC-2. The coding sequence and deduced amino acid sequences of the mature esterase protein are given in Table 9, and the purification of the Orpinomyces enzyme is described herein below. See also SEQ ID NOs: 17 and 18.

Brief Summary Text - BSTX (12):

A further aspect of the present invention methods for the recombinant production of the phenolic (especially **ferulic) acid esterases** of the present invention. Escherichia coli, Bacillus subtilis, Streptomyces sp., Saccharomyces cerevisiae, Aureobasidium pullulans, Pichia pastoris, Trichoderma, Aspergillus nidulans or any other host cell suitable for the production of a heterologous protein can be transfected or transformed with an expression vector appropriate for the chosen host. Compatible combinations of vectors and host cells are well known in the art as are appropriate promoters to be used to direct the expression of a particular coding sequence of interest. The recombinant host cells are cultured under conditions suitable for growth and expression of the **phenolic acid esterase** and the recombinant esterase is then collected or the recombinant host cells in which the esterase has been produced are collected. The coding sequence of the esterase can be operably linked to a nucleotide sequence encoding a signal peptide which is known in the art and functional in the desired host cell if secretion of the esterase into the culture medium is desired. In that case, the culture medium serves as the source of esterase after growth of the host cells.

Brief Summary Text - BSTX (13):

It is recognized by those skilled in the art that the DNA sequences may vary due to the degeneracy of the genetic code and codon usage. All DNA sequences which encode a **phenolic acid esterase** polypeptide having a specifically exemplified amino acid sequence are included in this invention, including DNA sequences encoding them having an ATG preceding the coding region for the mature protein and a translation termination codon (TAA, TGA or TAG) after the coding sequence.

Brief Summary Text - BSTX (14):

Additionally, it will be recognized by those skilled in the art that allelic variations may occur in the **phenolic acid esterase** polypeptide coding sequences which will not significantly change activity of the amino acid sequences of the polypeptides which the DNA sequences encode. All such equivalent DNA sequences are included within the scope of this invention and the definition of a **phenolic acid esterase**. The skilled artisan will understand that the amino acid sequence of an exemplified **phenolic acid esterase** polypeptide and signal peptide(s) can be used to identify and isolate additional, nonexemplified nucleotide sequences which will encode functional equivalents to the polypeptides defined by the amino acid sequences given herein or an amino acid sequence of greater than 40% identity thereto and having equivalent biological activity. All integer percents between 40 and 100 are encompassed by the present invention. DNA sequences having at least about 75% homology to any of the **ferulic acid esterases** coding sequences presented herein and encoding polypeptides with the same function are considered equivalent to thereto and are included in the definition of "DNA encoding a **phenolic acid esterase.**" Following the teachings herein, the skilled worker will be able to make a large number of operative embodiments having equivalent DNA sequences to those listed herein.

Brief Summary Text - BSTX (15):

The present invention encompasses **feruloyl esterase** proteins which are characteristic by at least a portion having from at least about 40% amino acid sequence identity with an amino acid sequence as given in SEQ ID NO:18, amino acids 227 to 440 (within the **feruloyl esterase** protein of Orpinomyces PC-2 of the present invention. All integer percent identities between 40 and 100% are also within the scope of the present invention. Similarly, the present invention encompasses **feruloyl esterase** proteins having from about 40% to about 100% identity with an amino acid sequence from the group comprising amino acids 581 to 789 of SEQ ID NO:16, amino acids 845 to 1075 of SEQ ID NO:12, amino acids 69 to 286 of SEQ ID NO:14, amino acids 69 to 307 of SEQ ID NO 14, and amino acids 69 to 421 of SEQ ID NO:14. Specifically exemplified **feruloyl esterases** of the present invention are characterized by amino acid sequences from the group comprising amino acids 227 to 440 of SEQ ID NO:18, amino acids 581 to 789 of SEQ ID NO:16, amino acids 845 to 1075 of SEQ ID NO:12, amino acids 69 to 286 of SEQ ID NO:14, amino acids 69 to 307 of SEQ ID NO:14, and amino acids 69 to 421 of SEQ ID NO:14. **Feruloyl esterase** proteins of the present invention include those having the following amino acid sequences: SEQ ID NO:18, amino acids 1 to 530; SEQ ID NO:12, amino acids 795 to 1077; SEQ ID NO:16, amino acids 546 to 789; SEQ ID NO:14, amino acids 20 to 286; SEQ ID NO:14, amino acids 20 to 307; and SEQ ID NO:14, amino acids 20 to 421.

Brief Summary Text - BSTX (16):

Specifically exemplified nucleotide sequences encoding the **feruloyl esterase** proteins of the present invention include the following: SEQ ID NO:17,

nucleotides 1 to 1590; SEQ ID NO:11, nucleotides 2582-3430; SEQ ID NO:15, nucleotides 2164 to 2895; SEQ ID NO:13, nucleotides 158 to 958; SEQ ID NO:13, nucleotides 158 to 1021; SEQ ID NO:13, nucleotides 158 to 1363.

Brief Summary Text - BSTX (17):

The **phenolic acid esterase** coding sequences, including or excluding that encoding a signal peptide of this invention, can be used to express a **phenolic acid esterase** of the present invention in recombinant fungal host cells as well as in bacteria, including without limitation, Bacillus spp., Streptomyces sp. and Escherichia coli. Any host cell in which the signal sequence is expressed and processed may be used. Preferred host cells are Aureobasidium species, Aspergillus species, Trichoderma species and Saccharomyces cerevisiae, as well as other yeasts known to the art for fermentation, including Pichia pastoris [See, e.g., Sreekrishna, K. (1993) In; Industrial Microorganisms: Basic and Applied Molecular Genetics, Baltz, R. H., et al. (Eds.) ASM Press, Washington, D.C. 119-126). Filamentous fungi such as Aspergillus, Trichoderma, Penicillium, etc. are also useful host organisms for expression of the DNA of this invention. [Van den Handel, C. et al. (1991) In: Bennett, J. W. and Lasure, L. L. (eds.), More Gene Manipulations in Fungi, Academy Press, Inc., New York, 397-428].

Drawing Description Text - DRTX (2):

FIG. 1 shows amino acid sequence alignment of the exemplified **phenolic acid esterases**. Sequences are xylanase Z [XynZ_Clotm, Grepinet et al. (1988) supra], xylanase Y [XynY_Clotm, Fontes et al. (1995) supra] of C. thermocellum, xylanase A (XynA_Rumin) of a Ruminococcus sp, and a hypothetical 44-kDa protein of E. coli (Genbank Accession Number P31471) (SEQ ID NO:19). Amino acid numbering was the same as in the databases. Dots represent gaps introduced to optimize alignment, and are treated as mismatched in calculations of sequence relatedness (similarity or identity). The partial amino acids are derived from SEQ ID NO:20, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO 19 and SEQ ID NO:18.

Drawing Description Text - DRTX (4):

FIG. 3 illustrates the results of Superose 6 gel filtration of proteins eluted from Avicel adsorption of C. thermocellum culture supernatant. Fractions (0.5 ml) were collected and assayed for protein and **feruloyl esterase** activity. Molecular mass standards (Sigma Chemical Company, St. Louis, Mo.) including blue dextran (2,000 kDa), catalase (232 kDa), ovalbumin (43 kDa), and ribonuclease A (13.7 kDa) were run under identical conditions and their elution positions were indicated.

Drawing Description Text - DRTX (7):

FIGS. 6A and 6B, respectively, illustrate the effects of temperature and pH on **feruloyl esterase** activity of the C. thermocellum XynZ FAE/CBD. Buffer used for evaluating temperature effects was 50 mM sodium citrate, pH 6.0. Assays

mixtures with a pH range from 2 to 10 were formulated by using a universal phosphate buffer system.

Drawing Description Text - DRTX (8):

FIG. 7 illustrates the results of SDS-PAGE analysis of the purified **feruloyl esterase** from the culture supernatant of Orpinomyces sp. strain PC-2 (lane 1); molecular mass markers are in lane 2.

Drawing Description Text - DRTX (9):

FIGS. 8A and 8B show the temperature and pH activity profiles, respectively, of the Orpinomyces sp. strain PC-2 **feruloyl esterase**.

Drawing Description Text - DRTX (10):

FIG. 9 shows alignment of protein sequences exhibiting homology to the Orpinomyces **feruloyl esterase**. Sequences are: faea_orpin, Orpinomyces sp. strain PC-2 FaeA; xyna_rumin, xylanase from Ruminococcus sp. (Genbank Accession Number S58235); yiel_ecoli hypothetical 44kDa protein from E. coli (Genbank Accession Number P31471); xyny_clotm, xylanase Y from C. thermocellum (Genbank Accession Number P51584); xynz_clotm, xylanase Z from C. thermocellum (Genbank Accession Number M22624); dppv_asprf, dipeptidyl peptidase from A. fumigatus (Genbank Accession Number L48074) (SEQ ID NO:20). The partial sequences are taken from SEQ ID NO:18, SEQ ID NO:16, SEQ ID NO:22, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:20.

Detailed Description Text - DETX (4):

Genes encoding **feruloyl esterase** (faeA) have been cloned from Aspergillus niger and Aspergillus tubingensis and the deduced amino acid sequences bear close similarity to lipases [de Vries et al. (1997) Appl. Environ. Microbiol. 63:4638-4644]. Expression of these gene products is regulated by the xlnR gene product [van Peij et al. (1998) Appl. Environ. Microbiol. 64:3615-3619]. Other genes include the xylD gene from Pseudomonas fluorescens subsp. cellulosa, the gene product of which has a higher specificity for acetyl groups than feruloyl groups [Ferreira et al. (1 993) Biochemical J. 294:349-355] and two genes from Butyrivibrio fibrisolvens termed cinA and cinB [Dalrymple and Swadling (1997) Microbiology 143:1203-1210; Dalrymple et al. (1996) FEMS Microbiol. Lett. 143:115-120]. These genes are believed to be regulated by the cinR gene product which may itself be regulated by FAXX [Dalrymple and Swadling (1997) supra]. Esterase activity has also been studied in Streptomyces olivochromogenes [Faulds and Williamson (1991) J. Gen. Microbiol. 137:2339-2345], Schizophyllum commune [MacKenzie and Bilous (1988) Appl. Environ. Microbiol. 54:1170-1173], Penicillium pinophillum [Castanares and Wood (1992) Biochem. Soc. Trans. 20:275S], and Fibrobacter succinogenes [McDermid et al. (1990) Appl. Environ. Microbiol. 56:127-132].

Detailed Description Text - DETX (5):

As described herein, **feruloyl esterases** are found as part of xylanases from the Clostridium thermocellum cellulosome or as an individual enzyme, for example, from Orpinomyces sp. PC-2. Xylanases Y and Z from C. thermocellum are composed of a xylanase domain, a linker domain, and other domains as well as a domain to which no function has been assigned. We found partial sequence homology between these enzyme and the **feruloyl esterase** of Orpinomyces in the region of the previously unknown domains and demonstrated that these domains indeed encode **feruloyl esterases**. Herein, we also report the purification, cloning, and partial characterization of the **feruloyl esterase** from Orpinomyces sp. strain PC-2.

Detailed Description Text - DETX (6):

Anaerobic fungi produce high levels of **phenolic esterases** [Borneman and Akin (1990) In: Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants. D. E. Akin, L. G. Ljungdahl, J. R. Wilson, and P. J. Harris (Eds.). Elsevier Science Publishing Co. New York, pp. 325-340] and two **feruloyl esterases** of the anaerobic fungus Neocallimastix MC-2 were purified and characterized [Borneman et al. (1992) Appl. Environ. Microbiol. 58:3762-3766]. A cDNA coding for a **feruloyl esterase** (FaeA) of the anaerobic fungus Orpinomyces PC-2 was cloned and sequenced by the present inventors. FASTA and BLAST searches showed that the catalytic domain of the Orpinomyces FaeA was over 30% identical to sequences coding for unknown domains (UD) in the databases including the carboxy terminal region of XynY Fontes et al. (1995) supra], the amino terminal region of XynZ [Grepinet et al. (1988) supra], a hypothetical polypeptide of E. coli (Genbank Accession Number P31471), and the carboxy terminal region of a Ruminococcus xylanase [Genbank Accession No. S58235] (FIG. 1). No function had been previously assigned to the sequences homologous to the Orpinomyces FaeA. XynY consists of multiple domains including a family F xylanase domain, followed by a putative thermostability domain, a dockerin, and the UD [Fontes et al. (1995) supra]. Similarly, XynZ is also multi-domain enzyme containing the UD, a family VI cellulose binding domain, a dockerin, and a family 10 xylanase domain [Grepinet et al. (1988) supra; Tomme et al. (1995) In: Enzymatic Degradation of Insoluble Carbohydrates. J. N. Saddler, M. H. Panner (Eds.), ACS Symposium Series, American Chemical Society, Washington, D.C., pp. 142-163]. Both XynY and XynZ are believed to be components of the cellulosome (FIG. 2). The Orpinomyces FaeA together with those homologous sequences, however, failed to show significant homology to the recently published **feruloyl esterases** (FaeA) of Aspergillus niger and A. tubingensis [de Vries et al. (1997) supra]. The sequence analysis implies that a new type of **feruloyl esterase** is encoded by the Orpinomyces cDNA and the homologous sequences described above.

Detailed Description Text - DETX (7):

We have determined that C. thermocellum produces **feruloyl esterase** activity under the conditions when the cellulosome production is induced. The bacterium was cultivated on low concentration (0.2%, w/v) of Avicel, and under this

growth condition, most of the substrate was consumed and cellulosomes released into culture medium, as indicated by the activities on Avicel and xylan (Table 2). Most of the **feruloyl esterase** activity (97.9%) was found in the culture medium (Table 2). It is well documented that cellulosomes of C. thermocellum are readily adsorbed to cellulose [Morag et al. (1992) Enzyme Microb. Technol. 14:289-292; Choi and Ljungdahl (1996) Biochemistry 35:4897-4905], and thus Avicel adsorption was used to assess association of the feruloyl activity with cellulosomes. As shown in Table 2,97.1% of total feruloyl activity was removed from the culture medium by Avicel treatment, even higher than the percentages of cellulase (80.5%) and xylanase (73.3%) activities removed. These data indicate that **feruloyl esterases** produced by C. thermocellum possess cellulose-binding ability through either a cellulose-binding domain or the cellulosomes. XynZ has a family VI cellulose binding domain [Grepinet et al. (1988) supra; Tonmme et al. (1995) supra] and a docking domain between the CBD and the dockerin, whereas XynY contains a docking domain.

Detailed Description Text - DETX (8):

Cellulosomes eluted from Avicel adsorption were analyzed by gel filtration chromatography using a Superose 6 column to assess the sizes of proteins containing **feruloyl esterase** activity in the native state. The majority of the proteins were eluted in fractions containing molecules with sizes around 2.0 million daltons (FIG. 3), characteristic of cellulosomes eluted from gel filtration [Choi and Ljungdahl (1996) supra]. **Feruloyl esterase** activity in the fractions correlated well with fractions of cellulosomes. No activity was found in fractions with protein molecules less than 200 kDa, indicating that **feruloyl esterase** activity resides in the cellulosome.

Detailed Description Text - DETX (9):

The UD coding region of XynY and various regions of XynZ were over-expressed in E. coli using the pRSET system (Invitrogen, Carlsbad, Calif.). Constructs spanning the XynY UD sequence, XynZ UD alone, and UD plus the CBD sequence in PRSET gave high levels of **feruloyl esterase** activity whereas cell-free extracts of E. coli harboring the pET-21b recombinant plasmid failed to hydrolyze FAXX. Constructs with 20 and 40 amino acid residues deleted from the C-terminus of the Xynz UD did not hydrolyze FAXX, indicating that XynZ sequence from the end of the signal peptide up to amino acid 288 was required to form an active **feruloyl esterase**. The heterologous protein band of the UD constructs without IPTG induction on SDS-PAGE analysis reached 40-50% of total protein. Both growth rates and levels of feruloyl activity of the constructs with the XynY and XynZ sequences were lower with IPTG induction than without induction. Without wishing to be bound by theory, it is believed that low level of T7 polymerase in E. coli BL21 (DE3) strain was ideal for the expression of the inserted genes in pRSET B, and over-expression of T7 polymerase gene by IPTG induction resulted in toxic levels of **feruloyl esterase** production.

Detailed Description Text - DETX (10):

Amino acid residues 328 to 419 of XynZ were homologous to two repeated CBDs

of C. stercorarium XynA [Sakka et al. (1993) supra; Sakka et al. (1995) supra] (FIG. 4). This domain has been recently classified as a family VI CBD [Tomme et al. (1995) supra]. Constructs containing the UD alone and both the UD plus the putative CBD of XynZ were purified from recombinant E. coli cultures. The majority of **feruloyl esterase** activity of the polypeptide containing both domains was removed by Avicel and acid swollen cellulose adsorption but not with the UD alone, indicating that strong cellulose binding capability resides in the family VI cellulose binding domain of XynZ. Cellulose-binding ability was confirmed with native gel retardation analysis.

Detailed Description Text - DETX (14):

In order to understand how microorganisms breakdown plant cell wall material, we chose to study enzymes from Clostridium thermocellum. In particular, XynY and XynZ from this organism were originally thought to contain a xylanase domain and second domain of unknown function. We have now demonstrated that the function of this domain is that of a **feruloyl esterase**. We believe this is the first report of a **phenolic acid esterase** in the cellulosome. **Feruloyl esterases** are important for the complete degradation of plant cell wall material. These enzymes are produced by several organisms, but they have not been found in a bifunctional enzyme.

Detailed Description Text - DETX (15):

A **feruloyl esterase** from Orpinomyces PC-2 was purified and internal fragments of the enzyme were used to screen the Orpinomyces PC-2 cDNA library. A partial clone was sequenced and showed homology to XynZ. A BLAST analysis showed that this enzyme, along with XynY, had domains of unknown function.

Detailed Description Text - DETX (16):

The high temperature stability of the enzyme is surprising because no other thermophilic **feruloyl esterases** have been reported until the present disclosure of the C. thermocellum thermotolerant **feruloyl esterases**. The Orpinomyces PC-2 enzyme has substrate specificity for both feruloyl and p-coumaroyl esterified substrates. The clostridial enzymes are the first from bacteria to have such a dual role. Although the Orpinomyces enzyme is not a true p-**coumaroyl esterase,** no p-**coumaric acid esterases** have been found in bacteria to date.

Detailed Description Text - DETX (18):

Feruloyl esterases and xylanase act synergistically to the release of ferulic acid and reducing sugars from lignocellulosic material [Borneman et al. (1993) supra]. In C. thermocellum XynY and Xynz, we hypothesize that this is more efficient due to the incorporation of both enzymes into one. We believe there is a multicutting event catalyzed by these enzymes much like the multicutting event in the cellulosome itself which leads to more efficient hydrolysis of plant cell wall material. The substrate, arabinoxylan could be

passed from one active site to another, which would eliminate the process of each of two enzymes having to bind to the substrate and then release it for the other enzyme to attack.

Detailed Description Text - DETX (19):

XynY and XynZ are enzymatic components of the Clostridium thermocellum cellulosome. These components have a multi-domain structure which includes a dockerin domain, a catalytic xylanase domain, and a domain of unknown function. The previously unknown domains in XynY and XynZ have been found to have **phenolic esterase** activity. These domains have some amino acid homology to that of a **phenolic esterase** from the anaerobic fungus Orpinomyces sp. strain PC-2. Secondly, purified cellulosomes from C. thermocellum hydrolyze (O-[5-O-[(E)-feruloyl]-(-L-arabinofuranosyl]-(1(3)-O-(-D-xylopyranosyl-(1(4)-D-xylopyranose) (FAXX) and [5-O-[(E)-feruloyl]-[O-(-D-xylopyranosyl-(1(2)]-O-(-L-arabinofuranosyl-[1(3]]-O-(-D-xylopyranosyl-1(4)-D-xylopyranose (FAX.sub.3) yielding ferulic acid as a product, thus indicating the presence of a **phenolic acid esterase**. Intracellular and extracellular fractions lacking cellulosomes had insignificant amounts of **phenolic acid esterase** activity which confirmed that the activity resided with the cellulosome. The final proof was obtained by cloning the domains of XynY and XynZ into Escherichia coli. The domains were expressed and found to possess **phenolic acid esterase** activities with FAXX and FAX.sub.3 as substrates.

Detailed Description Text - DETX (20):

Nucleotides corresponding to regions of DNA encoding amino acids in XynZ (Genbank Accession Number M22624) from 20-421 and in XynY (Genbank Accession Number X83269) from 795-1077 were overexpressed in E. coli using the pET and pRSET systems respectively. The XynZ sequence will henceforth be referred to as XynZ FAE/CBD since it incorporates the family VI CBD, and the XynY protein is XynY FAE since it only contains a catalytic domain. The cell free extracts containing the expressed proteins each hydrolyzed FAXX with release of ferulic acid (FA) which suggests that these proteins are **feruloyl esterases**. The expressed protein from the construct containing XynY FAE had a molecular weight of 31 kDa, consistent with the sequence data. Constructs containing XynZ FAE/CBD produced a protein with a molecular mass of 45 kDa as analyzed by SDS-PAGE. The protein was expressed without IPTG induction at a level of 8% of the total protein. Levels of **feruloyl esterase** activity of the constructs with the XynY FAE and XynZ FAE/CBD sequences were lower with IPTG induction than without induction. Since these proteins had similar sequences and similar function coupled with the fact that XynZ had higher expression levels than XynY, we decided to focus our attention on XynZ and subsequent experiments will refer to that protein.

Detailed Description Text - DETX (21):

Constructs were made which corresponded to proteins with amino acids from the original C. thermocellum XynZ sequence of 20-307 (FAE287), 20-286 (FAE) and

20-247 (FAE227) (with reference to SEQ ID NO. 14 and FIG. 2). FAE287 is missing the CBD, but contains a proline rich linker which separates the CBD from the FAE domain while FAE does not contain this linker. When these constructs were expressed in E. coli in the same manner as XynZ FAE/CBD, they both exhibited **feruloyl esterase** activity. Thus, the removal of the 114 amino acids of the C13D did not have a detrimental effect on the activity. XynlZ FAE/CBD bound to acid swollen cellulose very weakly, while the other constructs missing the CBD did not bind acid swollen cellulose at all. FAE227 was an inactive but expressed enzyme. The data here show that neither the CBD nor the linker is necessary for activity, but amino acids 247-266 are necessary for generation of an active enzyme. Since neither the linker region nor the CBD is necessary for activity, we used the smallest construct which still retained activity, FAE, for subsequent experiments.

Detailed Description Text - DETX (22):

The XynZ FAE/CBD polypeptide was purified from E. coli cell free extract after a single step of heat treatment at 70.degree. C. for 30 min. Over 200 mg of the XynZ FAE/CBD were obtained from 2.5 gram of crude protein (Table 3). The purified XynZ FAE/CBD had a mass as stated previously of 45 kDa as revealed by SDS-PAGE (FIG. 5), consistent with the calculated size (46.5 kDa). There was no evidence for aggregation of the **feruloyl esterase** produced in E. coli, and SDS-PAGE gels showed that protein which was removed from the cell free extract by centrifugation had no insoluble protein which could be attributed to inclusion bodies.

Detailed Description Text - DETX (25):

Anaerobic microorganisms do not readily degrade lignin, but are able to solubilize it. Anaerobic fungi are able to solubilize but not metabolize lignin, and it is suggested that the released lignin was carbohydrate linked [McSweeney et al. (1994) Appl. Environ. Microbiol. 60:2985-2989]. The data herein indicate that **feruloyl esterases** are responsible for lignin solubilization. Most studies of the cellulosome of C. thermocellum has been directed toward its cellulolytic activity. It also has xylanases which we have shown are bifunctional enzymes with **feruloyl esterase** activity. The cellulosome should be efficient in the degradation of arabinoxylan. It has been previously shown that Clostridium xylanolyticum released aromatics into the culture medium when grown on lignocellulosic material [Rogers et al. (1992) International Biodeterioration & Biodegradation 29:3-17].

Detailed Description Text - DETX (26):

XynY and XynZ each contain a glycosyl hydrolase family 10 catalytic domain in addition to the FAE catalytic domain. The xylanase domain of XynZ has been well studied, that construct has been crystallized, and the three dimensional structure solved [Dominguez et al. (1995) Nat. Struct. Biol. 2:569-576; Souchon et al. (1994) J. Mol. Biol. 235:1348-1350]. In general, xylanases are thought to be sterically hindered by groups substituted on the xylan backbone. **Feruloyl esterase** and xylanase have been shown to act synergistically for the

release of ferulic acid and reducing sugars from lignocellulosic material [Borneman et al. (1993) supra]. In XynY and XynZ we hypothesize that this event has been made more efficient by the incorporation of both FAE and xylanase catalytic domains into one enzyme. Without wishing to be bound by theory, we believe that there is a multicutting event catalyzed by these enzymes much like the multicutting event in the cellulosome itself which leads to more efficient hydrolysis of plant cell wall material. Bifunctional enzymes like XynY and XynZ form a dumbbell-like shape which attacks the arabinoxylan polysaccharide and the substrate is passed from one active site to another, eliminating the relatively inefficient two enzyme process in which one has to bind to the substrate and then release it for the other enzyme to attack. The existence of multidomain enzymes such as the sea whip coral peroxidase-lipoxygenase [Koljak et al. (1997) Science 277:1994-1996] and a xylanase-.beta.(1,3-1,4)-glucanase from Ruminococcus flavifaciens [Flint et al. (1993) J. Bacteriol. 175:2943-2951] suggests an evolutionary importance of having two or more catalytic domains in one enzyme. XynZ contains a contains a family VI CBD, which does not bind cellulose significantly. However, representatives of CBDs of this family usually efficiently bind xylan. The CBD of XynZ may participate in a tight association of the catalytic domains with the substrate. This is consistent with the higher Km of FAE as compared to that of XynZ FAE/CBD.

Detailed Description Text - DETX (28):

The FAE domains of XynZ and XynY are homologous to each other and to the Orpinomyces FaeA. The Orpinomyces FaeA, together with those homologous sequences, however, failed to show significant homology to the recently published **feruloyl esterases** (FaeA) of Aspergillus niger and A. tubingensis [de Vries et al. (1997) supra] as well as CinA and CinB from Butyrivibrio fibrisolvens [Dalrymple et al. (1996) FEMS Microbiol. Lett. 143:115-120; Dalrymple and Swadling (1997) Microbiology 143:1203-1210] and XylD from Pseudomonas fluorescens subsp. cellulosa [Ferreira et al. (1993) Biochemical Journal 294:349-355]. The sequence analysis implies that a new type of **feruloyl esterase** is encoded by the Orpinomyces gene and the homologous C. thermocellum sequences described above. The Orpinomyces FaeA, and the FAE domains of XynZ and XynY were also shown to be homologous to a hypothetical polypeptide of E. coli (Genbank Accession Number P31471) and the carboxy terminal region of a Ruminococcus sp. xylanase earlier designated as a UD [Genbank Accession Number S58235]. No function had been assigned to those sequences of E. coli and Ruminococcus. Without wishing to be bound by theory, the present inventors believe that these sequences also encode **feruloyl esterases** and that the Ruminococcus xylanase is also bifunctional. Ruminococcus has been shown to produce FAE activity [McSweeney et al. (1998) Anaerobe 4:57-65], and another Ruminococcus xylanase has been shown to be a bifunctional enzyme with xylanase and acetyl xylan esterase activity [Kirby et al. (1998) Biochemical Society Transactions 26:S169]. No **feruloyl esterase** activity has been observed in E. coli. The gene from E. coli may encode a dipeptidase instead, because homology exists between a dipeptidase from Aspergillus fumigatus and **feruloyl esterases**. The data suggest a common ancestral encoding **feruloyl esterases** from Orpinomyces, C. thermocellum, and Ruminococcus.

Detailed Description Text - DETX (30):

The **feruloyl esterase** domain of XynZ was highly expressed in E. coli and the esterase comprised 40-50% of the total cell protein. The recombinant esterase of XynZ was purified to almost homogeneity by heat treatment. The protein had a molecular mass of 45 kDa, consistent with the size of the predicted deduced amino acid sequence. Of the substrates tested, the expressed protein had high specific activity towards FAXX and FAX.sub.3. With FAX.sub.3 as a substrate Km and Vmax values were 3.2 mM and 13.5 .mu.mol ferulic acid released mind mg-l respectively at pH 6.0 at 60.degree. C. Several phenolic esterified substrates were hydrolyzed and the specific activities with those containing feruloyl groups were higher than were those with p-coumaroyl groups confirming that the previously unknown domain of XynZ is a **feruloyl esterase**. The enzyme released mainly ferulic acid from wheat bran and Coastal Bermuda grass (CBG) with a smaller amount of p-coumaroyl groups released from CBS. This study represents the first demonstration of esterases in the cellulosome of Clostridium thermocellum and of enzymes from the cellulosome with two different activities. The present work also provides a **phenolic acid esterase** derived from a xylanase from Ruminococcus and as an enzyme produced by Orpinomyces PC-2.

Detailed Description Text - DETX (31):

A summary of the purification of FAE from Orpinomyces sp stain PC-2 is presented in Table 7. The Q-Sepharose column separated two peaks of esterase activity. Proteins which eluted in the first peak had higher activity against ethyl-pCA while proteins eluting in the second peak had greater activity against FAXX. These data suggest that a p**-coumaroyl esterase** eluted in the first peak while the **feruloyl esterase** eluted in the second. The first peak was not studied further, but the fractions in peak 2 were further purified resulting in a purified enzyme which had an approximate molecular mass of 50 kDa as visualized by SDS-PAGE analysis (FIG. 7). There was a decrease in specific activity after the MonoQ step which could not be explained.

Detailed Description Text - DETX (33):

Two of the peptide fragments from the internal amino acid sequencing were used to create degenerate aglionucleotide primers which are listed in the materials and methods section. These primers were used to amplify regions of DNA in the Orpinomyces PC-2 cDNA library. A 216 bp PCR product was generated. The PCR product was labeled with digoxygenin-UTP and used as a probe to screen the cDNA library. After screening 50,000 phage, one positive plaque was obtained and its DNA was sequenced using T3 and T7 universal primers. Sequencing using the T3 primer did not reveal any ORFs, however, sequencing using the T7 reverse primer gave the C-terminal end of the gene. Based on the sequence data and restriction fragment analyses, but without wishing to be bound by theory, we have concluded that the faeA gene in this cDNA was truncated and furthermore that the insert comprises multiple genes. These other genes were not studied further. The deduced amino acid sequence of the insert matched the data from the peptide sequencing. The insert had a size of 1074 bp and encoded a protein of 358 amino acids. Since the size of the

encoded protein did not match that of the purified enzyme and the N-terminal sequence, including a signal peptide and lack of a start codon, another round of screening was performed using the entire sequence as a probe after digoxygenin labeling. After screening an additional 50,000 phage, one positive clone was obtained which had a size of 1673 bp with the largest open reading frame comprising a protein of 530 amino acids. The sequence of this insert is believed to be an incomplete one since no 5' UTR was found and the (putative) signal sequence has only four amino acids. Most signal sequences found in hydrolytic enzymes from anaerobic fungi are at least 20 amino acids long. The insert was found to be in a reverse orientation with respect to the LacZ promoter. The upstream lac promoter should direct synthesis of the inserted gene, but no activity was found in lysed E. coli cells harboring the recombinant plasmid. The FaeA gene in E. coli was expressed using the pET system (Novagen) in the correct orientation. The recombinant FaeA released ferulic acid from FAXX as well as other substrates which were esterified with phenolic groups. The enzyme had the highest activity against FAXX, which demonstrates that it is a true **feruloyl esterase** (Table 10). In addition, when the enzyme was incubated with a recombinant xylanase, there was a 80 fold increase in FA released over FaeA alone.

Detailed Description Text - DETX (35):

It will be understood by those skilled in the art that other nucleic acid sequences besides those disclosed herein for the **phenolic acid esterases, i.e. feruloyl esterases,** will function as coding sequences synonymous with the exemplified coding sequences. Nucleic acid sequences are synonymous if the amino acid sequences encoded by those nucleic acid sequences are the same. The degeneracy of the genetic code is well known to the art. For many amino acids, there is more than one nucleotide triplet which serves as the codon for a particular amino acid, and one of ordinary skill in the art understands nucleotide or codon substitutions which do not affect the amino acid(s) encoded. It is further understood in the art that codon substitutions to conform to common codon usage in a particular recombinant host cell is sometimes desirable

Detailed Description Text - DETX (36):

Specifically included in this invention are sequences from other strains of Clostridium and from other microorganisms which hybridize to the sequences disclosed for **feruloyl and coumaryl esterases** under stringent conditions. Stringent conditions refer to conditions understood in the art for a given probe length and nucleotide composition and capable of hybridizing under stringent conditions means annealing to a subject nucleotide sequence, or its complementary strand, under standard conditions (i.e., high temperature and/or low salt content) which tend to disfavor annealing of unrelated sequences, (indicating about 95-100% nucleotide sequence identity). Also specifically included in this invention are sequences from other strains of Orpinomyces species and other anaerobic fungi which hybridize to the sequences disclosed for the esterase sequences under moderately stringent conditions. Moderately stringent conditions refer to conditions understood in the art for a given probe sequence and "conditions of medium stringency" means hybridization and

wash conditions of 50.degree.-65.degree. C., 1.times.SSC and 0.1% SDS (indicating about 80-95% similarity). Also specifically included in this invention are sequences from other strains of Orpinomyces, from other anaerobic fungi, and from other organisms, including bacteria, which hybridize to the sequences disclosed for the esterase sequences under highly stringent conditions. Highly stringent conditions refer to conditions understood in the art for a given probe sequence and "conditions of high stringency" means hybridization and wash conditions of 65.degree.-68.degree. C., 0.1.times.SSC and 0.1% SDS (indicating about 95-100% similarity). Hybridization assays and conditions are further described in Sambrook et al. (1989).

Detailed Description Text - DETX (37):

A method for identifying other nucleic acids encoding **feruloyl esterase**- and/or coumaryl esterase-homologous enzymes is also provided wherein nucleic acid molecules encoding **phenolic acid esterases** are isolated from an anaerobic fungus, including but not limited to Orpinomyces or an anaerobic bacterium, such as Clostridium or Ruminococcus, among others, and nucleic acid hybridization is performed with the nucleic acid molecules and a labeled probe having a nucleotide sequence that includes all or part of a FAE coding sequence as given in Table 5, 6, 9 and/or 10 herein. By this method, **phenolic acid esterase** genes similar to the exemplified **feruloyl and coumaryl esterases** can be identified and isolated from other strains of Clostridium or other anaerobic microorganisms. All or part of a nucleotide sequence refers specifically to all continuous nucleotides of a nucleotide sequence, or e.g. 1000 continuous nucleotides, 500 continuous nucleotides, 100 continuous nucleotides, 25 continuous nucleotides, and 15 continuous nucleotides.

Detailed Description Text - DETX (38):

Sequences included in this invention are those amino acid sequences which are 40 to 100% identical to the amino acid sequences encoded by the exemplified C. thermocellum strain **feruloyl esterase,** amino acids proteins truncated from the XynY or XynZ proteins or the Ruminococcus FAE polypeptide or the Orpinomyces PC-2 FAE polypeptide, all specifically identified herein. Sequences included in this invention are also those amino acid sequences which are 40, 50, 60, 70, 75, 80, 85, 90, 95 to 100%, and all integers between 40% and 100%, identical to the amino acid sequences encoded by an exemplified **phenolic acid esterase** coding sequence and corresponding to or identifying encoded proteins which exhibit **feruloyl esterase** activity. In comparisons of protein or nucleic acid sequences, gaps introduced into either query or reference sequence to optimize alignment are treated as mismatches. In amino acid sequence comparisons to identify **feruloyl esterase** proteins, the reference sequence is, desirably, amino acids 227 to 440 of SEQ ID NO:18 (FAE of Orpinomyces PC-2).

Detailed Description Text - DETX (40):

Monoclonal or polyclonal antibodies, preferably monoclonal, specifically

reacting with the **phenolic acid esterases** of the present invention may be made by methods known in the art. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratories; Goding (1986) Monoclonal Antibodies: Principles and Practice, 2d ed., Academic Press, New York.

Detailed Description Text - DETX (50):

Genomic DNA was isolated from C. thermocellum as previously described [Maniatis et al. (1982) supra]. PCR primers were designed (Table 1) and synthesized on an Applied Biosystems (Foster City, Calif.) DNA sequencer. To facilitate the insertion of DNA sequence into or pET-21b or pRSET B, BamHI (for pET-216) or NdeI for pRSET B, and HindIII sites were added to forward and reverse primers, respectively (Table 1). PCRs were carried out on a Perkin Elmer 480 Thermocycler for 30 cycles with each cycle on 95.degree. C. for 1 min, 48.degree. C. for 1 min, and 72.degree. C. for 3 min. PCR products and the plasmid were digested with BamHI (or NdeI) and HindIII, purified with a Bio101 Geneclean kit, ligated with T4 ligase. E. coli BL21 (DE3) was transformed with the ligation mixture and at least four colonies of each construct were picked for analyzing **feruloyl esterase** expression. The inserted sequences were sequenced to verify the lack of unwanted mutations.

Detailed Description Text - DETX (51):

Two internal sequences were used to create degenerate oligonucleotide primers for PCR in order to amplify the **feruloyl esterase** coding sequence in the cDNA library in Orpinomyces. The Orpinomyces PC-2 cDNA library is described in the .lambda.ZAPII vector (Stratagene, La Jolla, Calif.) in E. coli host cells is described in Chen et al. (1995) Proc. Natl. Acad. Sci. 92:2587-2591. Positive clone(s) are subcloned a pBluescript vector (Stratagene, La Jolla, Calif.). The amplified product was cloned into pCRII (Invitrogen, Carlsbad, Calif.) using the TA cloning kit and sequenced using an automatic PCR sequencer (Applied Biosystems, Foster City, Calif.) using M13 reverse primer. The resulting PCR product was used to screen the cDNA library after being labeled with digoxigenin (Boehringer Mannheim, Indianapolis, Ind.). The digoxigenin probe was bound to plaques which were lifted from a nitrocellulose blot. Antibodies conjugated to alkaline phosphatase showed a single positive clone which hybridized to the PCR product. The product was sequenced and found to contain the C-terminal 358 amino acids of the enzyme (See Table 9). A second probe which incorporated those 339 amino acids was used as a probe to screen the library in the same manner as before. A second clone was isolated which contained the C-terminal region plus an additional 172 amino acids making a polypeptide of 530 amino acids. Confirmation of the sequence came from N-terminal and internal protein sequence data from the purified enzyme which matched that of the cloned cDNA product. Expression cloning of this coding sequence, which lacks an ATG translation start site, can be achieved by expressing it, in frame, as a fusion protein using any one of a number of fusion protein vectors known to the art or an ATG translation start codon and/or ribosome binding site upstream of the ATG can be added using methodology well known to and readily accessible to the art in an expression vector appropriate to the choice of recombinant host cell.

Detailed Description Text - DETX (54):

The cellulosomes were isolated from 10L of culture fluid after complete substrate exhaustion by the affinity digestion method [Morag et al. (1992) supra]. This preparation was used directly for gel filtration using a Fast Protein Liquid Chromatography (FPLC) system with a Superose 6 column (Pharmacia, Piscataway, N.J.). Proteins were eluted in 50 mM Tris-HCl, 100 mM NaCl at a flow rate of 0.2 ml/min. Fractions of 0.5 ml were collected and stored at 4.degree. C. for further analysis. Cell extracts were prepared by first growing the organism in the presence of 0.2% cellobiose for 2 days. Cells were then separated by centrifugation, resuspended in 50 mM Tris-HCl buffer, pH 7.5, and sonicated. Culture medium was concentrated to 5 ml using a Millipore concentrator (Millipore, Bedford, Mass.). To adsorb cellulosomes from the medium, 0.5 mg of Avicel was added and the suspension was stirred at 4.degree. C. for 4 hours. Avicel was removed by centrifugation (Avicel-treated medium). All fractions were tested for Avicelase, xylanase, and **ferulic acid esterase** activities.

Detailed Description Text - DETX (55):

Unless otherwise noted, all C. thermocellum enzyme assays were performed at 60.degree. C. in 50 mM Na-citrate buffer, pH 6.0. One unit of enzyme activity was defined as the amount of enzyme that released 1 .mu.mol of product min-1, and specific activity is given in units per milligram of protein. **Feruloyl esterase** activity was measured using a modified version of the assay described by Borneman et al. [Borneman et al. (1990) Anal. Biochem. 190:129-133]. The appropriately diluted protein sample (25 l) was added to 400 .mu.l of buffer plus 8 mM of substrate. Samples were incubated at 60.degree. C. for 5 min. and the reaction was stopped by adding 25 .mu.l of 20% formic acid. Release of ferulic acid was measured via HPLC using a mobile phase of 10 mM Na-formate pH 3 and 30% (vol/vol) methanol. For routine assays, FAXX and FAX3 purified from wheat bran were used as substrates [Borneman et al. (1990) supra]. Ethyl-ferulate and ethyl-p-coumarate esters were a gift from D. E. Akin (USDA, Athens, Ga.). The hydrolysis of these (10 mM) were determined similarly to that of FAXX, but the HPLC analyses were performed with 50% methanol. HPLC runs were with a Hewlett Packard 1100 Series instrument equipped with an autosampler and diode array detector. Ferulic acid and p-coumaric acid were used as standards. To determine the amount of feruloyl and p-coumaroyl groups released from plant cell walls, wheat bran and Coastal Bermuda grass were ground in a Wiley mill to pass through a 250 .mu.m screen. Plant samples of ten milligram were incubated for one hour in 400 .mu.l of 50 mM Na-citrate buffer pH, 6.0 plus 25 .mu.l of enzyme. After the addition of 25 .mu.l of 20% formic acid to stop the reaction, the samples were centrifuged at 16,000.times.g in a microfuge and then assayed for FA and pCA by HPLC.

Detailed Description Text - DETX (57):

Unless otherwise noted, all Orpinomyces enzyme assays were performed at 40.degree. C. in 50 mM Bis-Tris Propane buffer, pH 6.0. One unit of enzyme activity is defined as the amount that released 1 .mu.mol of product min-1, and

specific activity is given in units per milligram of protein. Protein was determined by the method of Bradford [Bradford, M. (1976) Anal. Biochem. 72:248-254]. **Feruloyl esterase** activity was assayed by the method of Borneman et al. [(1990) supra] which involved measuring the release of ferulic acid from FAXX via HPLC using a mobile phase of 10 mM Na-formate pH 3 and 30% (vol/vol) methanol. FAXX was purified from wheat bran as previously described [Borneman et al. (1990) supra]. For assay using ethyl-p-coumarate (ethyl-pCA), the substrate (10 mM) was used with 30% methanol in the same mobile phase. Samples were run on a Hewlett Packard 1100 Series instrument equipped with an autosampler and diode array detector. Ferulic acid and p-coumaric acid were used as standards. The appropriately diluted protein sample (25 .mu.l) was added to 400 .mu.l of buffer containing 750 .mu.M FAXX. Samples were incubated at 40.degree. C. for 30 min. and the reaction was stopped by adding 25 .mu.l of 20% formic acid. pH optimum assays were carried out in 100 mM citrate phosphate buffer in the range of 2.6-7.0, 100 mM phosphate in the range of pH 5.7-6.3, and 100 mM Tris in the range of pH 7.0-9.0. For temperature optimum determination, purified esterase were incubated for 30 minutes at the appropriate temperature within the range of 20.degree. to 70.degree. C.

Detailed Description Text - DETX (63):

A **feruloyl esterase** was purified from culture supernatant of Orpinomyces sp. strain PC-2 (Barichievicz and Calza medium [Barichievicz and Calza (1990) Appl. Environ. Microbiol. 56:43-48] with 0.2% Avicel as carbon source). The enzyme was obtained from a 60 liter culture of the fungus. The culture was grown under an atmosphere of CO.sub.2 for 6 days. The fungal mycelia were removed by filtration through Miracloth (Calbiochem, San Diego, Calif.) The culture supernatant was concentrated 120 fold using a Pellicon system (Millipore, Bedford, Mass.) and a 10 kDa membrane. The concentrate was loaded onto a Q Sepharose (Pharmacia, Piscataway, N.J.) column equilibrated with 20 mM Tris.multidot.HCl pH 7.5, and proteins were eluted with a gradient of 1 M NaCl in the same buffer. The active fractions were detected by their ability to release ferulic acid from FAXX as measured by HPLC. The active fractions were combined and ammonium sulfate was added to a concentration of 1.7M. The solution was filtered and then loaded onto a Phenyl Sepharose High Performance Chromatography (Pharmacia) column equilibrated with 20 mM Tris.multidot.HCl pH 7.5 and 1.7 M ammonium sulfate. The protein was eluted by a negative gradient of buffer without ammonium sulfate. Active fractions were concentrated using a Centricon 10 unit (Amicon, Millipore, Bedford, Mass.) and subsequently applied to a TSK 3000SW column (Tosohaas, Montgomeryville, Pa.) which was equilibrated with 20 mM Tris.multidot.HCl pH 7.5 and 200 mM NaCl. Fractions with activity were combined and loaded directly onto an anion exchange (MonoQ HR 5/5, Pharmacia, Piscataway, N.J.) column equilibrated with 20 mM Tris.multidot.HCl pH 7.5. The purified enzyme was eluted using a gradient of 0.5 M NaCl. The purification is summarized in Table 7.

Detailed Description Text - DETX (75):

Table 9 presents the deduced amino acid sequence and cDNA coding sequence of the mature **phenolic acid esterase** of Orpinomyces PC-2.

Detailed Description Text - DETX (76):

FIG. 1 provides the amino acid sequence for a **phenolic acid esterase (feruloyl esterase)** which corresponds to a previously uncharacterized Ruminococcus xylanase. The sequence of the complete coding sequence of that xylanase is available under Accession No. S58235 (Gen Bank)(See Table 10). The coding sequence of the **phenolic acid esterase** polypeptide is nucleotide 2164-2895, exclusive of translation start and stop codons.

Detailed Description Paragraph Table - DETL (1):

TABLE 1 Primer used in amplifying various regions of xynY and xynZ of C. thermocellum Name Sequence.sup.a Gene Direction Position.sup.b SEQ ID NO: XYF1Bam.sup.a1 TAGGATCCCCTGTAGCAGAAAATCCTTC xynY Forward 795-800 1 XYF1.sup.c
TACATATGCCTGTAGCAGAAAATCCTTC xynY Forward 795-800 2 XYR1.sup.c
GAGGAAGCTTTTACATGGAAGAAATATGGAAG xynY Reverse 1071-1077 3 XZF1.sup.d
TACATATGCTTGTCACAATAAGCAGTACA xynZ Forward 20-26 4 XZF1Bam
TAGGATCCCTTGTCACAATAAGCAGTACA xynZ Forward 20-26 5 XZR1.sup.d
GAGGAAGCTTTTAGTTGTTGGCAACGCAATA xynZ Reverse 242-247 6 XZR2.sup.d
GAGGAAGCTTACTTCCACACATTAAAATC xynZ Reverse 261-266 7 XZR3.sup.d
GAGGAAGCTTAGTTTCCATCCCTCGTCAA xynZ Reverse 281-286 8 XZR4.sup.d
GAGGAAGCTTAGTCATAATCTTCCGCTTC xynZ Reverse 302-307 9 XZR5.sup.d
GAGGAAGCTTAAACGCCAAAAGTGAACCAGTC xynZ Reverse 414-421 10 .sup.a Restriction sites NdeI and HindIII are underlined and double-underlined, respectively. .sup.a1 Restriction site BamH1 is underlined. .sup.b Amino acid positions are according to xylanase sequences in the data banks. .sup.c XYF1 or XYF1Bam and XYR1 are the forward and reverse primers used to amplify the **feruloyl esterase** domain from xylY(xynY) of C. thermocellum [see Fontes et al. (1995) supra]. .sup.d XZFI is the forward primer and XZR1-XZR5 are the reverse primers used in the amplification of the **feruloyl esterase** portion of the xynZ of C. thermocellum.

Detailed Description Paragraph Table - DETL (5):

TABLE 4 Substrate specificity of the **feruloyl esterase** in C. thermocellum XynZ. Substrate Specific activity (U/mg) FAXX 12.5 FAX.sub.3 11.8 PAX.sub.3 1.4.sup.a Ethyl-FA 0.066 Ethyl-pCA 0.022 CMC 0 PNP-arabinopyranoside 0 PNP-glucopyranoside 0 PNP-xylopyranoside 0 Wheat bran 0.06 Coastal Bermuda grass 0.1 .sup.a Calculated value based on substrate concentration used in the assay

Detailed Description Paragraph Table - DETL (8):

TABLE 7 Purification of a **Feruloyl Esterase** from Orpinomyces PC-2 Culture Supernatant Total Total Activity Protein Specific Ac- Purification Step (U) (mg) tivity (Umg.sup.-1) Fold Culture Supernatant 32.38 5,830 5.6E - 3 1 Concentrate 7.9 1460 5.42E - 3 0.96 Q Sepharose 2.58 181 1.43e - 2 2.55 Phenyl Sepharose 1.68 28.2 5.96E - 2 10.6 HP TSK 3000 SW 0.85 0.62 1.39 253

Mono Q HR 5/5 0.26 0.24 1.087 198

Detailed Description Paragraph Table - DETL (9):

TABLE 7 Purification of a **Feruloyl Esterase** from Orpinomyces PC-2 Culture Supernatant Total Total Activity Protein Specific Ac- Purification Step (U) (mg) tivity (Umg.sup.-1) Fold Culture Supernatant 32.38 5,830 5.6E - 3 1 Concentrate 7.9 1460 5.42E - 3 0.96 Q Sepharose 2.58 181 1.43e - 2 2.55 Phenyl Sepharose 1.68 28.2 5.96E - 2 10.6 HP TSK 3000 SW 0.85 0.62 1.39 253 Mono Q HR 5/5 0.26 0.24 1.087 198

Claims Text - CLTX (1):

1. A recombinant DNA molecule comprising a vector sequence and a sequence encoding a **feruloyl esterase protein, wherein said feruloyl esterase** protein is characterized by an amino acid sequence having at least 75% amino acid sequence identity with amino acids 227 to 440 of SEQ ID NO:18.

Claims Text - CLTX (2):

2. The recombinant DNA molecule of claim 1, wherein said **feruloyl esterase** protein is characterized by the amino acid sequence given in amino acids 227 to 440 of SEQ ID NO:18.

Claims Text - CLTX (3):

3. The recombinant DNA molecule of claim 2, wherein the **feruloyl esterase** comprises the amino acid sequence given in SEQ ID NO:18, amino acids 5 to 530.

Claims Text - CLTX (4):

4. The recombinant DNA molecule of claim 3, wherein the sequence encoding the **feruloyl esterase** protein comprises the sequence given in SEQ ID NO:17, nucleotides 13 to 1590.

Claims Text - CLTX (5):

5. The recombinant DNA molecule of claim 3, wherein the **feruloyl esterase** comprises the sequence given in SEQ ID NO:18, amino acids 1 to 530.

Claims Text - CLTX (6):

6. The recombinant DNA molecule of claim 5, wherein the sequence encoding the **feruloyl esterase** protein comprises the sequence given in SEQ ID NO:17, nucleotides 1 to 1590.

Claims Text - CLTX (8):

8. The recombinant host cell of claim 7, wherein said **feruloyl esterase** protein is characterized by the amino acid sequence given as amino acids 227 to 440 of SEQ ID NO:18.

Claims Text - CLTX (9):

9. The recombinant host cell of claim 8 wherein the **feruloyl esterase** comprises the amino acid sequence given in SEQ ID NO:18, amino acids 5 to 530.

Claims Text - CLTX (10):

10. The recombinant host cell of claim 9 wherein the sequence encoding the **feruloyl esterase** protein comprises the sequence given in SEQ ID NO:17, nucleotides 13 to 1590.

Claims Text - CLTX (11):

11. The recombinant host cell of claim 9 wherein the **feruloyl esterase** comprises the sequence given in SEQ ID NO:18, amino acids 1 to 530.

Claims Text - CLTX (12):

12. The recombinant host cell of claim 11, wherein the sequence encoding the **feruloyl esterase** protein comprises the sequence given in SEQ ID NO:17, nucleotides 1 to 1590.

Claims Text - CLTX (13):

13. A recombinant DNA molecule comprising a vector sequence and a sequence encoding a **feruloyl esterase protein, wherein the feruloyl esterase** protein consists of an amino acid sequence selected from the group consisting of amino acids 581 to 789 of SEQ ID NO:16, amino acids 346 to 789 of SEQ ID NO:16, amino acids 795 to 1077 of SEQ ID NO:12, amino acids 20 to 286 of SEQ ID NO:14, amino acids 20 to 307 of SEQ ID NO:14, and amino acids 20 to 421 of SEQ ID NO:14.

Claims Text - CLTX (14):

14. The recombinant DNA molecule of claim 13, wherein the **feruloyl esterase** consists of an amino acid sequence as given in SEQ ID NO:12, amino acids 795 to 1077.

Claims Text - CLTX (15):

15. The recombinant DNA molecule of claim 14, wherein the sequence encoding the **feruloyl esterase** protein is given in SEQ ID NO:11, nucleotides 2582-3430.

Claims Text - CLTX (16):

16. The recombinant DNA molecule of claim 13, wherein the **feruloyl esterase** consists of the amino acid sequence as given in SEQ ID NO:16, amino acids 546 to 789.

Claims Text - CLTX (17):

17. The recombinant DNA molecule of claim 16, wherein the sequence encoding the **feruloyl esterase** protein is given in SEQ ID NO:15, nucleotides 2164 to 2895.

Claims Text - CLTX (18):

18. The recombinant DNA molecule of claim 13, wherein the **feruloyl esterase** consists of an amino acid sequence as given in SEQ ID NO:14, amino acids 20 to 286.

Claims Text - CLTX (19):

19. The recombinant DNA molecule of claim 18, wherein the sequence encoding the **feruloyl esterase** protein is given in SEQ ID NO:13, nucleotides 158 to 958.

Claims Text - CLTX (20):

20. The recombinant DNA molecule of claim 13, wherein the **feruloyl esterase** consists of the amino acid sequence given in SEQ ID NO:14, amino acids 20 to 307.

Claims Text - CLTX (21):

21. The recombinant DNA molecule of claim 20, wherein the sequence encoding the **feruloyl esterase** protein is given in SEQ ID NO:13, nucleotides 158 to 1021.

Claims Text - CLTX (22):

22. The recombinant DNA molecule of claim 13, wherein the **feruloyl esterase** consists of the amino acid sequence given in SEQ ID NO:14, amino acids 20 to 421.

Claims Text - CLTX (23):

23. The recombinant DNA molecule of claim 22, wherein the sequence encoding the **feruloyl esterase** protein is given in SEQ ID NO:13, nucleotides 158 to 1363.

Claims Text - CLTX (25):

25. A method for the recombinant production of a **feruloyl esterase** protein comprising the step of culturing a recombinant host cell comprising a vector sequence and a sequence encoding a **feruloyl esterase protein, wherein the feruloyl esterase** protein consists of an amino acid sequence selected from the group consisting of amino acids 581 to 789 of SEQ ID NO:16, amino acids 795 to 1077 of SEQ ID NO:12, amino acids 20 to 286 of SEQ ID NO:14, amino acids 20 to 307 of SEQ ID NO:14, amino acids 20 to 421 of SEQ ID NO:14, amino acids 5 to 530 of SEQ ID NO:18 and an amino acid sequence of at least 75% amino acid sequence identity with amino acids 227 to 440 of SEQ ID NO:18, under conditions of nutrition, time and temperature such that a **feruloyl esterase** protein is produced via expression of the sequence encoding the **feruloyl esterase** protein contained within the recombinant DNA molecule within said host cell.

Claims Text - CLTX (26):

26. The method of claim 25, wherein the **feruloyl esterase** protein consists of an amino acid sequence from the group consisting of amino acids 581 to 789 of SEQ ID NO:16, amino acids 795 to 1077 of SEQ ID NO:12, amino acids 845 to 1075 of SEQ ID NO:12, amino acids 20 to 286 of SEQ ID NO:14, amino acids 20 to 307 of SEQ ID NO:14, amino acids 20 to 421 of SEQ ID NO:14, amino acids 1 to 530 of SEQ ID NO:18, and amino acids 5 to 530 of SEQ ID NO:18.

Other Reference Publication - OREF (1):

Blum et al. (1999) "Characterization of a **Feruloyl Esterase** from the Anaerobic Fungus Orpinomyces sp. Strain PC-2" Abstracts. 99.sup.th General Meeting of the American Society for Microbiology. Chicago, IL. May 30-Jun. 3, 1999. vol. 99, pp. 430-431.

Other Reference Publication - OREF (3):

Borneman et al. (1992) "Purification and Partial Characterization of Two **Feruloyl Esterases** from the Anaerobic Fungus Neocallimastix Strain MC-2" Applied and Environmental Microbiology 58:3762-3766.*

Other Reference Publication - OREF (4):

Borneman et al. (1990) "Assay for trans-p-**Coumaroyl Esterase** Using a Specific Substrate from Plant Cell Walls" Analytical Biochemistry 190:129-133.*

Other Reference Publication - OREF (5):

Castanares and Wood (1992) "Purification and Characterization of a Feruloyl/p-**Coumaroyl Esterase** from Solid-State Cultures of the Aerobic Fungus Penicillium pinophilum" Biochemical Society Transactions 20:275S.*

Other Reference Publication - OREF (10):

De Vries et al. (1997) "The faeA Genes from Aspergillus niger and Aspergillus tubingensis Encode **Ferulic Acid Esterases** Involved in Degradation of Complex Cell Wall Polysaccharides" Applied and Environmental Microbiology 63:4638-4644.

Other Reference Publication - OREF (11):

Faulds and Williamson (1991) "The Purification and Characterization of 4-Hydroxy-3-Methoxycinnamic (**Ferulic) Acid Esterase** from Streptomyces olivochromogenes" Journal of General Microbiology 137:2339-2345.

Other Reference Publication - OREF (25):

MacKenzie and Bilous (1988) "**Ferulic Acid Esterase** Activity from Schizophyllum commune" Applied and Environmental Microbiology 54:1170-1173.

Other Reference Publication - OREF (27):

McSweeney et al. (1998) "Butyrivibrio spp. and Other Xylanolytic Microorganisms from the Rumen have **Cinnamoyl Esterase** Activity" Anaerobe 4:57-65.

US-PAT-NO: 6361808

DOCUMENT-IDENTIFIER: US 6361808 B1

TITLE: Process for the production of alcoholic beverages using maltseed

DATE-ISSUED: March 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Souppe; Jerome	Wasquehal	N/A	N/A	FR
Beudeker; Robert Franciscus	Den Hoorn	N/A	N/A	NL

APPL-NO: 09/ 230590

DATE FILED: April 28, 1999

PARENT-CASE:

This application is a 371 of PCT/EP97/04016, filed Jul. 23, 1997.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	96202195	August 5, 1996

PCT-DATA:
APPL-NO: PCT/EP97/04016
DATE-FILED: July 23, 1997
PUB-NO: WO98/05788
PUB-DATE: Feb 12, 1998
371-DATE: Apr 28, 1999
102(E)-DATE:Apr 28, 1999

US-CL-CURRENT: 426/29

ABSTRACT:

The invention relates to a process for the production of alcoholic beverages such as beer or whiskey with a mixture of enzymes comprising an endo-.beta.(1,4)-xylanase, an arabinofuranosidase, an alpha-amylase, an endo-protease and a .beta.-(1,3, 1,4)-glucanase, and optionally, a saccharifying amylase and/or an exopeptidase. Preferable are mixtures in which the enzymes which are necessary in the brewing process are provided by transgenic seeds. Only a minor amount of malt may be necessary for flavor and color.

9 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Detailed Description Text - DETX (6):

Hemicellulolytic enzymes comprise enzymes like .beta.-1,3-1,4-glucanase, xylanase, endo-arabinanase, arabinofuranosidase, arabinoxylanase, arabinogalactanase, **ferulic acid esterase**.

US-PAT-NO: 6323011

DOCUMENT-IDENTIFIER: US 6323011 B1

TITLE: Production of vanillin

DATE-ISSUED: November 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Narbad; Arjan	Norfolk	N/A	N/A	GB
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Gasson; Michael John	Norfolk	N/A	N/A	GB
Walton; Nicholas John	Norfolk	N/A	N/A	GB

APPL-NO: 09/ 155183

DATE FILED: May 3, 1999

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9606187	March 23, 1996

PCT-DATA:
APPL-NO: PCT/GB97/00809
DATE-FILED: March 24, 1997
PUB-NO: WO97/35999
PUB-DATE: Oct 2, 1997
371-DATE: May 3, 1999
102(E)-DATE:May 3, 1999

US-CL-CURRENT: 435/147

ABSTRACT:

A method of producing vanillin comprising the steps of: (1) providing trans-ferulic acid or salt thereof; and (2) providing trans-ferulate; CoASH ligase activity (enzyme activity I) trans-feruloyl SCoA hydratase activity (enzyme activity II), and 4-hydroxy-3-methoxyphenyl-.beta.-hydroxy-propionyl SCoA (HMPHP SCoA) cleavage activity (enzyme activity III). Conveniently the enzymes are provided by Pseudomonas fluorescens Fe3 or a mutant or derivative thereof. Polypeptides with enzymes activities II and III and polynucleotides encoding said polypeptides. Use of said polypeptides or said polynucleotides in a method for producing vanillin.

19 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 27

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Brief Summary Text - BSTX (50):

Trans-ferulic acid or a salt thereof is readily available from plant material. Suitably, trans-ferulic acid or a salt thereof is released from the plant material by the action of **ferulic acid esterase**. Thus, in a particularly preferred embodiment of the invention the trans-ferulic acid or salt thereof is provided by the action of **ferulic acid esterase** on plant material.

Brief Summary Text - BSTX (52):

Thus, advantageously the trans-ferulic acid or a salt thereof may be provided by the action of trans-**ferulic acid esterase** on said ester. More particularly, it is advantageous to introduce a gene encoding said esterase into a host cell or organism which is being used in the methods of the invention. Thus, it is convenient to introduce a trans-**ferulic acid esterase** gene, such as the aforementioned XYLD gene, into a plant which is being used in the methods of the invention.

Claims Text - CLTX (15):

11. A method according to claim 1 wherein the trans-ferulic acid or salt thereof is provided by action of trans-**ferulic acid esterase** on plant material, said plant material containing an ester of trans-ferulic acid.

Other Reference Publication - OREF (30):

Faulds et al., "Release of Ferulic Acid from Wheat Bran by a **Ferulic Acid Esterase** (FAE-III) from Aspergillus niger" Appl. Microbiol. Biotechnol., 43:1082-1087 (1995).

Other Reference Publication - OREF (32):

Faulds et al., "The Purification and Characterization of 4-Hydroxy-3-Methoxycinnamic (**Ferulic) Acid Esterase** form Streptomyces olivochromogenes," J. Gen. Microbiol., 137:2339-2345 (1991).

Other Reference Publication - OREF (33):

Castanares et al., "Pruification and Properties of a Feruloyl/.rho.-**Coumaroyl Esterase** from the Fungus Penicillium phiophilum." Enzyme Microb. Technol., 14:875-884 (1992).

Other Reference Publication - OREF (34):

Borneman et al., "Feruloyl and p-**Coumaroyl Esterase** from Anaerobic Fungi in Relation to Plant Cell Wall Degradation," Appl. Microbiol. Biotechnol., 33:345-351 (1990).

Other Reference Publication - OREF (35):

MacKenzie et al., "**Ferulic Acid Esterase** Activity from Schizophyllum commune," Appl. Environ. Microbiol., 54:1170-1173 (1988).

Other Reference Publication - OREF (36):

Tenkanen et al., "Production, Purification and Characterization of an **Esterase Liberating Phenolic** Acids from Lignocellulosics," J. Biotechnol., 18:69-84 (1991).

Other Reference Publication - OREF (37):

Faulds et al., "Purification and Characterization of a **Ferulic Acid Esterase** (FAE-III) from Aspergillus niger: Specificity for The Phenolic Moiety and BInding to Microcrystalline Cellulose," Microbiol., 140:779-787 (1994).

US-PAT-NO: 6303106

DOCUMENT-IDENTIFIER: US 6303106 B1

TITLE: Allomelanin production

DATE-ISSUED: October 16, 2001

INVENTOR-INFORMATION:

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APPL-NO: 09/ 077912

DATE FILED: September 25, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9524997	December 7, 1995
GB	9525428	December 13, 1995

PCT-DATA:
APPL-NO: PCT/GB96/03015
DATE-FILED: December 9, 1996
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PUB-DATE: Jun 12, 1997
371-DATE: Sep 25, 1998
102(E)-DATE:Sep 25, 1998

US-CL-CURRENT: 424/62, 424/401 , 424/450 , 514/568 , 514/569 , 514/944

ABSTRACT:

A method of producing a melanin comprises oxidizing a phenolic compound at one or more hydroxyl groups thereof, wherein the phenolic compound is selected from 5-hydroxyindole and derivatives thereof and compounds of formula (1) and the oxidation is provided by biotransformation in the presence of an oxidoreductase enzyme, the compound of formula (1), ##STR1##

wherein R.sup.1 is H or OH; R.sup.2 is H, OH orOCH.sub.3 ; R.sup.3 is H or OH at least one of R.sup.1 and R.sup.3 being OH; R.sup.4 is selected from H , R, --COOX and R.sup.7 --COOX, wherein R is an optionally substituted saturated or unsaturated alkyl group having from 1 to 12 carbon atoms, R.sup.7 is an optionally substituted saturated or unsaturated alkylene group having from 1 to 12 carbon atoms and X is selected from H and aliphatic and aromatic ester forming groups; and R.sup.5 and R.sup.6 is each independently selected from H, OH, NH.sub.2, OCH.sub.3, CH.sub.3, SH, NHCO.sub.2, NHCH.sub.3, COOH and saturated or unsaturated alkyl groups having up to 8 carbon atoms.

32 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (37):

For example ferulic acid compounds, ie. ferulic acid and derivatives thereof, may be prepared by a direct enzyme conversion of one or more compounds present in the plant cells. For example, wheat germ added to water may be treated by the enzyme preparations derived from microbial strains such as Humicola insolens that produce enzyme preparations containing **ferulic acid esterase** such as the enzyme Celluzyme.TM. (as described in a copending PCT patent application No. PCT/GB96/01345 by the present applicants) or Biofeed.TM. to produce ferulic acid directly. Alternatively, the ferulic acid compound may be prepared by enzyme conversion of one or more compounds present in plant cells to produce an intermediate such as caffeic acid or a derivative thereof followed by further treatment of the intermediate to produce the required ferulic acid compound. Preparation of caffeic acid compounds by enzyme treatment of compounds such as chlorogenic acid present in plant cells and tissues is described in our pending International Patent Application PCT/GB95/01324 and is further described on a further copending UK Patent Application of even date by the present applicants. The caffeic acid compound prepared thereby may be converted into the required ferulic acid compound by o-methylation of one of the hydroxyl groups of caffeic acid compound. This conversion step may be carried out chemically but is preferably carried out biochemically. For example, we have found that addition of the micro-organism Streptomyces griseus NRRL 8090 to caffeic acid compounds provides the required selective conversion.

US-PAT-NO: 6300112

DOCUMENT-IDENTIFIER: US 6300112 B1

TITLE: .beta.-xylosidase, nucleotide sequence encoding it, and use thereof

DATE-ISSUED: October 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
De Graaff; Leendert Hendrik	Oosterbeek		N/A	NL
Van Peij; Noel Nicolaas Maria Elisabeth	Wageningen		N/A	NL
	Bennekom	N/A	N/A	NL
Van Den Broeck; Henrietta Catharina	Wageningen		N/A	NL
Visser; Jacob				

APPL-NO: 08/ 981446

DATE FILED: December 22, 1997

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	95201707	June 23, 1995

PCT-DATA:
APPL-NO: PCT/NL96/00258
DATE-FILED: June 24, 1996
PUB-NO: WO97/00964
PUB-DATE: Jan 9, 1997
371-DATE: Dec 22, 1997
102(E)-DATE:Dec 22, 1997

US-CL-CURRENT: 435/195, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

A nucleotide sequence is provided which encodes a peptide having .beta.-xylosidase activity and exhibits at least 30% amino acid identity with the amino acid sequence shown in SEQ ID NO. 1 and described in SEQ ID NO. 3 or hybridises under stringent conditions with a nucleotide sequence shown in SEQ ID NO. 1, or a part thereof having at least 15 nucleotides encoding an amino acid sequence shown in SEQ ID NO. 1 and described in SEQ ID NO. 3. Also provided is a peptide having .beta.-xylosidase activity and exhibiting at least 30% amino acid identity with the amino acid sequence shown in SEQ ID NO. 1 and described in SEQ ID NO. 3 or a part thereof having at least 8 amino acids shown in SEQ ID NO. 1 and described in SEQ ID NO. 3.

4 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Brief Summary Text - BSTX (23):

The invention is furthermore concerned with the use of regulatory sequences contained in the 5'-noncoding part of SEQ ID NO.1 (nucleotides 1-854 or a part thereof) for expression of homologous or heterologous genes, e.g. xylanase, amylase, glucanase, oxidoreductases e.g. hexose oxidase, .alpha.-glucuronidase, lipase, **esterase, ferulic acid esterase,** proteases, or human interleukin-6, bovine (pro)chymosin, human lactoferrin, fungal phytase. A signal sequence of xlnD may be used in such constructs, as well as a suitable terminator, e.g. xlnD or trpC.

Brief Summary Text - BSTX (25):

The host cell--whether altered so as to produce or overproduce .beta.-xylosidase or so as not to produce its .beta.-xylosidase--may advantageously express or overexpress other relevant proteins, including enzymes, in particular other xylanolytic enzymes such as endoxylanases, and/or other enzymes such as amylases, glucanases, oxidoreductases such as hexose oxidase, .alpha.-glucuronidase, lipases, **esterases, ferulic acid esterase** and/or proteases. The corresponding genes may be under the control of homologous control regions or under the control region of the .beta.-xylosidase gene contained in the nucleotide sequence described above.

US-PAT-NO: 6245363

DOCUMENT-IDENTIFIER: US 6245363 B1

TITLE: Methods of treating plant materials with hydrolytic enzymes isolated from humicola species

DATE-ISSUED: June 12, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Myers; Stephen John	Ashford	N/A	N/A	GB
Cheetham; Peter S.J.	Turnbridge Wells	N/A	N/A	GB
Banister; Nigel E.	London	N/A	N/A	GB

APPL-NO: 08/ 973948

DATE FILED: March 31, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
WO	PCT/GB95/01324	June 7, 1995
GB	9524353	November 29, 1995

PCT-DATA:
APPL-NO: PCT/GB96/01345
DATE-FILED: June 7, 1995
PUB-NO: WO96/39859
PUB-DATE: Dec 19, 1996
371-DATE: Mar 31, 1998
102(E)-DATE:Mar 31, 1998

US-CL-CURRENT: 426/2, 426/132 , 426/53 , 426/615 , 426/623 , 426/629 , 426/630 , 426/635 , 426/807

ABSTRACT:

The invention relates to methods for treating plant material such as sunflower seed meal with water and a hydrolytic enzyme in an aqueous environment to increase its nutritional value and to enzymatic methods for converting plant derived material into useful products.

24 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (7):

Faulds, C. and Williamson, G., "Release of ferulic acid from wheat bran by a **ferulic acid esterase** (FAE-III) from aspergillus niger"; Applied Microbiology and Biotechnology, pp. 1082-1087, vol. 43, no. 6, 1995.

US-PAT-NO: 6235507

DOCUMENT-IDENTIFIER: US 6235507 B1

TITLE: Microbiological process for producing vanillin

DATE-ISSUED: May 22, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Muheim; Andreas	Zurich	N/A	N/A	CH
Muller; Bruno	Dubendorf	N/A	N/A	CH
Munch; Thomas	Illnau	N/A	N/A	CH
Wetli; Markus	Forch	N/A	N/A	CH

APPL-NO: 09/ 096230

DATE FILED: June 11, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	97110010	June 19, 1997

US-CL-CURRENT: 435/147, 435/123 , 435/155

ABSTRACT:

A microbiological process for producing vanillin and other useful products from ferulic acid is disclosed. Vanillin and guaiacol are recovered from the process using a differential pH extraction where the guaiacol is recovered at a pH of greater than 9 and the vanillin is recovered at a pH of from about 5 to about 8.

8 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Brief Summary Text - BSTX (9):

Ferulic acid as a substrate for biotransformations is abundantly available from different natural sources. The acid often occurs in the form of a glucoside in plant materials, such as wood, sugar beet melasse, bran of corn, rice and various types of grasses. It can be isolated from the corresponding glycosides in these products by well-known hydrolysis methods, e.g. using

enzymes, and can be used as crude material or purified material. A British source (GB 2301103 A1) describes for instance the enzymatic breakdown of ferulic acid containing plant material by a **ferulic acid esterase,** in order to obtain the free acid.

US-PAT-NO: 6232101

DOCUMENT-IDENTIFIER: US 6232101 B1

TITLE: Oxidase-promoted gelling of phenolic polymers

DATE-ISSUED: May 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Budolfsen; Gitte	Frederiksberg	N/A	N/A	DK
Heldt-Hansen; Hans Peter	Virum	N/A	N/A	DK

APPL-NO: 08/ 732260

DATE FILED: October 28, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a 35 U.S.C. 371 national application of PCT/DK95/00317 filed Jul. 26, 1995, which is incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0882/94	July 26, 1994

PCT-DATA:
APPL-NO: PCT/DK95/00317
DATE-FILED: July 26, 1995
PUB-NO: WO96/03440
PUB-DATE: Feb 8, 1996
371-DATE: Oct 28, 1996
102(E)-DATE:Oct 28, 1996

US-CL-CURRENT: 435/72, 424/488 , 435/101 , 435/274 , 435/275 , 435/99 , 514/54 , 536/123.1 , 536/126 , 536/128

ABSTRACT:

A method for causing gelling or increase of viscosity of an aqueous medium containing a gellable polymeric material having substituents with phenolic hydroxy groups comprises adding an oxidase, particularly a laccase, to the aqueous medium.

21 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (23):

The phenolic-substituted cinnamic acid ester linkages can be hydrolysed by **ferulic acid esterases**. Enzymes used in the purification of polysaccharides containing substituents of the cinnamic acid type should therefore be essentially free from **ferulic acid esterase** activity with specificity towards ferulic acid esters of the polysaccharide in question. Under conditions of low water activity, **ferulic acid esterase** will catalyse the formation of new ester linkages to carbohydrates, and can therefore be used to increase the content of ester residues of the phenolic cinnamic acid ester type (e.g. feruloyl residues) in cereal arabinoxylan and pectin from beet (or other members of the Chenopodiaceae) and thereby their gelling properties.

Detailed Description Text - DETX (24):

Polysaccharides (and other types of polymers) which do not contain phenolic residues useful for achieving gelation can be derivatized in order to render them gellable. Under conditions of low water activity, **ferulic acid esterases** can be used to attach groups of the cinnamic acid ester type (e.g. ferulic acid ester groups) to polymers such as pectin, arabinan, galactan, cellulose derivatives (e.g. hydroxyethylcellulose or carboxymethylcellulose), galactomannans (e.g. guar gum, hydroxypropyl-guar gum or locust bean gum), beta-glucans, xyloglucans, starch, derivatized starch, bacterial gums (e.g. xanthan), algal gums (e.g. alginate or carrageenan), other polysaccharides or other polymers with hydroxyl groups.

US-PAT-NO: 6218167

DOCUMENT-IDENTIFIER: US 6218167 B1

TITLE: Stable biocatalysts for ester hydrolysis

DATE-ISSUED: April 17, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Allen; Larry	Northfield	IL	N/A	N/A
Aikens; John	LaGrange Park	IL	N/A	N/A
DeMirjian; David	Chicago	IL	N/A	N/A
Vonstein; Veronika	Chicago	IL	N/A	N/A
Fonstein; Michael	Chicago	IL	N/A	N/A
Casadaban; Malcolm	Chicago	IL	N/A	N/A

APPL-NO: 09/ 058260

DATE FILED: April 10, 1998

PARENT-CASE:

This application is a continuation-in-part of U.S. Ser. No. 08/827,810 filed Apr. 11, 1997 (abandoned) which is a continuation-in-part of U.S. Ser. No. 08/781,802 filed Jan. 10, 1997 (now U.S. Pat. No. 5,969,121) which is a continuation-in-part of U.S. Ser. No. 08/694,078 filed Aug. 8, 1996 (pending) which claims priority to U.S. Ser. No. 60/019,580 filed Jun. 12, 1996 and U.S. Ser. No. 60/009,704 filed Jan. 11, 1996.

US-CL-CURRENT: 435/252.3, 435/196 , 435/252.33 , 435/320.1 , 536/23.2

ABSTRACT:

The instant invention encompasses isolated stable esterase enzymes characterized by the ability to remain stable at certain temperatures, substrate specificities, and activity profile; the expression vectors which can express, nucleic acids which encode for, and corresponding protein amino acid sequence of such proteins.

4 Claims, 60 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 55

---------- KWIC ---------

Detailed Description Text - DETX (185):

11. Faulds, C. B. and G. Williamson. (1993) **Ferulic Acid Esterase** from Aspergillus niger--Purification and Partial Characterization of 2 Forms from a Commercial Source of Pectinase. Biotechnol Appl Biochem. 17:349-359.

US-PAT-NO: 6218163

DOCUMENT-IDENTIFIER: US 6218163 B1

TITLE: Stable biocatalysts for ester hydrolysis

DATE-ISSUED: April 17, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Allen; Larry	Northfield	IL	N/A	N/A
Aikens; John	LaGrange Park	IL	N/A	N/A
Demirjian; David	Chicago	IL	N/A	N/A
Vonstein; Veronika	Chicago	IL	N/A	N/A
Fonstein; Michael	Chicago	IL	N/A	N/A
Casadaban; Malcolm	Chicago	IL	N/A	N/A

APPL-NO: 08/ 694078

DATE FILED: August 8, 1996

PARENT-CASE:

This application claims priority to U.S. Provisional Applications for patent Ser. No. 06/019,580, filed Jun. 12, 1996; Ser. No. 60/009,704, filed Jan. 11, 1996; and Ser. No. 60/001,995, filed Aug. 7, 1995, all of which are hereby incorporated by reference in their entirety.

US-CL-CURRENT: 435/197, 435/196 , 435/252.3 , 435/320.1 , 435/826 , 435/832 , 435/839 , 435/849 , 530/350 , 536/23.2

ABSTRACT:

The instant invention encompasses isolated stable esterase enzymes characterized by the ability to remain stable at certain temperatures, substrate specificities, and activity profile.

3 Claims, 60 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 53

---------- KWIC ---------

Detailed Description Text - DETX (138):

11. Faulds, C. B. and G. Williamson. (1993) **Ferulic Acid Esterase** from

Aspergillus niger--Purification and Partial Characterization of 2 Forms from a Commercial Source of Pectinase. Biotechnol Appl Biochem. 17:349-359.

US-PAT-NO: 6177261

DOCUMENT-IDENTIFIER: US 6177261 B1

TITLE: Method to isolate mutants and to clone the complementing gene

DATE-ISSUED: January 23, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
De Graaff; Leendert Hendrik	Oosterbeek		N/A	N/A	NL
Van Den Broeck; Henrietta Catharina	Bennekom		N/A	N/A	NL
Visser; Jacob	Wageningen	N/A	N/A	NL

APPL-NO: 08/ 981729

DATE FILED: December 23, 1997

PARENT-CASE:

This application is a 371 Of PCT/NL96/00259 filed Jun. 24, 1996.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	9520107	June 23, 1995
EP	95202346	August 30, 1995

PCT-DATA:
APPL-NO: PCT/NL96/00259
DATE-FILED: June 24, 1996
PUB-NO: WO97/00962
PUB-DATE: Jan 9, 1997
371-DATE: Dec 23, 1997
102(E)-DATE:Dec 23, 1997

US-CL-CURRENT: 435/69.1, 435/254.11 , 435/320.1 , 536/23.74 , 536/24.1

ABSTRACT:

The subject invention lies in the field of microorganism mutation and selection of the mutants. In particular, the invention is directed at obtaining metabolic mutants in a simple, direct and specific manner. In a preferred embodiment it is also possible to obtain desired mutants not comprising recombinant DNA, thereby facilitating incorporation thereof in products for human consumption or application, due to shorter legislative procedures. The method according to the invention involves random mutation and specific selection of the desired metabolic mutant. A nucleic acid cassette comprising a nucleic acid sequence encoding a bidirectional marker, said

nucleic acid cassette further comprising a basic transcriptional unit operatively linked to the nucleic acid sequence encoding the bidirectional marker and said nucleic acid cassette further comprising an inducible enhancer or activator sequence linked to the basic transcription unit in such a manner that upon induction of the enhancer or activator sequence the bidirectional marker encoding nucleic acid sequence is expressed, said inducible enhancer or activator sequence being driven from a gene associated with metabolism is claimed as is application thereof in a selection method for mutants. In addition a regular gene xInR encoding an activating regulator of an inducible enhancer or activator sequence and application of said gene and/or its expression product in overexpression of homologous or heterologous protein or peptide is described. Knockout mutants wherein said gene is absent or inactivated and mutants with increased or decreased DNA binding capacity are also claimed.

32 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

Detailed Description Text - DETX (92):

Genes of particular interest for expressing using the expression cassette according to the invention or in combination with a nucleic acid sequence according to the invention are those encoding enzymes. Suitable genes fore expressing are genes encoding xylanases, glucanases, oxidoreductases such as hexose oxidase, .alpha.-glucuronidase, lipase, **esterase, ferulic acid esterase** and proteases. These are non limiting examples of desirable expression products. A number of sequences are known in the state of the art comprising the genes mentioned and such information is readily available to the person skilled in the art and is to be considered incorporated herein. The genes can either be readily synthesized on the basis of known sequences in the literature or databases or be derived from organisms or vectors comprising them in a standard manner known per se and are considered to be knowledge readily available to the person skilled in the art not requiring further elucidation.

US-PAT-NO: 6143543

DOCUMENT-IDENTIFIER: US 6143543 A

TITLE: Enzyme system comprising **ferulic acid esterase** from Aspergillus

DATE-ISSUED: November 7, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Michelsen; Birgit	Frederiksberg	N/A	N/A	DK
De Vries; Ronald Peter	Wageningen	N/A	N/A	NL
Visser; Jacob	Wageningen	N/A	N/A	NL
S.o slashed.e; J.o slashed.rn	Mundelstrup	N/A	N/A	DK
Borch	Braband	N/A	N/A	DK
Poulsen; Charlotte Horsmans	Aarhus	N/A	N/A	DK
Zargahi; Masoud R.				

APPL-NO: 08/ 975600

DATE FILED: November 21, 1997

US-CL-CURRENT: 435/196, 435/187 , 435/189 , 435/195 , 435/197

ABSTRACT:

An enzyme system is described that is useful for preparing food and feed. One enzyme of that system is obtainable from Aspergillus. That enzyme has the following characteristics: a MW of from 29 kDa to 36 kDa as measured on a SDS-Phastgel (10-15%) or about 30 kDa; a pI value of about 3-4; **ferulic acid esterase** activity; a pH optimum of about 5; and a temperature optimum of from about 50 to about 60.degree. C.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Abstract Text - ABTX (1):

An enzyme system is described that is useful for preparing food and feed. One enzyme of that system is obtainable from Aspergillus. That enzyme has the following characteristics: a MW of from 29 kDa to 36 kDa as measured on a SDS-Phastgel (10-15%) or about 30 kDa; a pI value of about 3-4; **ferulic acid esterase** activity; a pH optimum of about 5; and a temperature optimum of from about 50 to about 60.degree. C.

TITLE - TI (1):

Enzyme system comprising **ferulic acid esterase** from Aspergillus

Brief Summary Text - BSTX (3):

In particular, the enzyme system of the present invention comprises a **ferulic acid esterase** ("FAE"). More in particular, the present invention relates to a nucleotide sequence coding for an FAE and the FAE itself.

Brief Summary Text - BSTX (18):

According to a first aspect of the present invention there is provided an enzyme system comprising a **ferulic acid esterase** ("FAE") and at least one protein or enzyme of interest ("POI"), wherein the FAE comprises any one or more of the sequences shown as SEQ. I.D. No. 1, SEQ. I.D. No. 2, SEQ. I.D. No. 3, SEQ. I.D. No. 6, or SEQ I.D. No. 7, or a variant, homologue or fragment thereof, preferably wherein if the FAE is genomic FAE and if just one POI is present then that POI is not a xylanase.

Brief Summary Text - BSTX (22):

c. **ferulic acid esterase** activity

Brief Summary Text - BSTX (30):

c. **ferulic acid esterase** activity

Brief Summary Text - BSTX (39):

c. **ferulic acid esterase** activity

Brief Summary Text - BSTX (43):

According to a fifth aspect of the present invention there is provided a recombinant enzyme having **ferulic acid esterase** activity, the enzyme comprising any one or more of the sequences shown as SEQ. I.D. No. 1, SEQ. I.D. No. 2, SEQ. I.D. No. 3, SEQ. I.D. No. 6, or SEQ. I.D. No. 7, or a variant, homologue or fragment thereof.

Brief Summary Text - BSTX (44):

According to a sixth aspect of the present invention there is provided a recombinant enzyme having **ferulic acid esterase** activity and encoded by a

nucleotide sequence comprising any one or more of the sequences shown as SEQ. I.D. No. 4, SEQ. I.D. No. 5, or SEQ. I.D. No. 6, or a variant, homologue or fragment thereof or a sequence complementary thereto.

Brief Summary Text - BSTX (51):

According to a thirteenth aspect of the present invention there is provided a recombinant **ferulic acid esterase** enzyme, which is immunologically reactive with an antibody raised against a purified **ferulic acid esterase** enzyme which comprises at least one of the sequences shown as SEQ. I.D. No. 1, SEQ. I.D. No. 2, SEQ. I.D. No. 3, or SEQ. I.D. No. 7.

Brief Summary Text - BSTX (88):

c. **ferulic acid esterase** activity

Brief Summary Text - BSTX (95):

c. **ferulic acid esterase** activity

Brief Summary Text - BSTX (100):

The advantages of the present invention are that it provides a means for preparing an enzyme having **ferulic acid esterase** activity and a nucleotide sequence coding for the same. The enzyme is particularly useful for preparing food and feed, in particular dough and bakery products. In this regard, the enzyme of the present invention can do any one or more of: hydrolyse wheat, release species that can act as an anti-oxidant and preferably as an in situ anti-oxidant, release species that can act can stabilise an oxidising agent or enzyme and preferably stabilise in situ an oxidising agent or enzyme, and it can be used to increase nutrient absorption. The FAE of the present invention may even impair or prevent the formation of Form III horse radish peroxidase ("HRP") and preferably it can impair or prevent the in situ formation of Form III HRP.

Brief Summary Text - BSTX (114):

The present invention therefore provides an enzyme having **ferulic acid esterase** activity wherein the enzyme can be prepared by recombinant DNA techniques, such as expression of a gene coding for the same in certain or specific cells or tissues, such as in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the genus Aspergillus, such as Aspergillus niger. The enzyme may even be prepared by a plant. The FAE may be used alone or in combination with one or more other proteins or enzymes.

Brief Summary Text - BSTX (119):

The terms "variant", "homologue" or "fragment" in relation to the enzyme include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acid from or to the sequence providing the resultant amino acid sequence has **ferulic acid esterase** activity, preferably having at least the same activity of the enzyme comprising at least one of the sequences shown in the sequence listings (SEQ. I.D. No. 1, SEQ. I.D. No. 2, SEQ. I.D. No. 3, or SEQ. I.D. No. 7). In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant enzyme has **ferulic acid esterase** activity. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to an enzyme comprising at least one of the sequences shown in the sequence listings (SEQ. I.D. No. 1, SEQ. I.D. No. 2, SEQ. I.D. No. 3, SEQ. I.D. No. 6, or SEQ. I.D. No. 7). More preferably there is at least 95%, more preferably at least 98%, homology to the sequences shown in the attached sequence listings. The same commentary is equally applicable to the terminal sequence.

Brief Summary Text - BSTX (120):

The terms "variant", "homologue" or "fragment" in relation to the nucleotide sequence coding for the enzyme include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence codes for an enzyme having **ferulic acid esterase** activity, preferably having at least the same activity of the enzyme comprising at least one of the sequences shown in the sequence listings (SEQ. I.D. No. 1, SEQ. I.D. No. 2, SEQ. I.D. No. 3, or SEQ. I.D. No. 7). In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence codes for an enzyme having **ferulic acid esterase** activity. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to a nucleotide sequence comprising any one or more of the sequences shown as SEQ. I.D. No. 4, SEQ. I.D. No. 5, or SEQ. I.D. No. 6. More preferably there is at least 95%, more preferably at least 98%, homology to the sequences shown in the attached sequence listings.

Brief Summary Text - BSTX (193):

c. **ferulic acid esterase** activity

Brief Summary Text - BSTX (201):

c. **ferulic acid esterase** activity

Brief Summary Text - BSTX (207):

In summation, the present invention therefore provides an enzyme having **ferulic acid esterase** activity and a nucleotide sequence coding for the same.

In addition the present invention provides an enzyme system comprising FAE.

Claims Text - CLTX (1):

1. An enzyme system comprising an isolated purified **ferulic acid esterase** (FAE) and at least one enzyme of interest (EOI), wherein the FAE comprises the amino acid sequences of SEQ ID NOs: 1 and 2.

Claims Text - CLTX (2):

2. An enzyme system comprising an isolated and purified **ferulic acid esterase** (FAE) and at least one enzyme of interest (EOI), wherein the FAE comprises the amino acid sequences of SEQ ID NOs: 1, 2 and 3.

Claims Text - CLTX (7):

7. An isolated and purified **ferulic acid esterase** (FAE) comprising the amino acid sequences of SEQ ID NOs: 1 and 2.

Claims Text - CLTX (8):

8. An isolated and purified **ferulic acid esterase** (FAE) comprising the amino acid sequences of SEQ ID NOs: 1, 2 and 3.

Other Reference Publication - OREF (1):

de Vries et al. "The faeA genes from Aspergillus niger and Aspergillus tubingensis encode **ferulic acid esterase** involved in degradation of complex cell wall polysaccharides" App. Environ. Microbiol. 63 (12), 4638-4644, Dec. 1997.

Other Reference Publication - OREF (2):

Faulds et al. "Purification and characterization of a **ferulic acid esterase** (FAE-III) from Aspergillus niger: specificity for the phenolic moity to microcrystalline cellulose" Microbiology 140, 799-787, 1994.

Other Reference Publication - OREF (3):

Faulds et al. "**Ferulic acid esterase** from Aspergillus niger: purification and partial characterization of two forms from commercial source of pectinase" Biotechnol. App. Biochem. 17, 349-359, Jun. 1993.

Other Reference Publication - OREF (5):

Ralet et al. "Degradation of feryloylated oligosaccharides from sugar-beet pulp and wheat bran by **ferulic acid esterase** from Aspergillus niger" Carbohyd. Res. 263, 257-269, 1994.

Other Reference Publication - OREF (6):

Faulds et al. "Release of ferulic acid from plant polysaccharides by **ferulic acid esterase** from Streptomyces olivochromogenes," Carbohydrate Polymers 21:153-55 (1993).

US-PAT-NO: 5998176

DOCUMENT-IDENTIFIER: US 5998176 A

TITLE: Gelling of pectic material using carboxylic ester hydrolase and oxidase and/or peroxidase

DATE-ISSUED: December 7, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Budolfsen; Gitte	Frederiksberg	N/A	N/A	DK
Pedersen; Lars Saaby	Farum	N/A	N/A	DK

APPL-NO: 09/ 085344

DATE FILED: May 27, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of PCT/DK97/00037 filed on Jan. 27, 1997 and claims priority under 35 U.S.C. 119 of Danish application serial no. 0092/96 filed Jan. 26, 1996, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0092/96	January 26, 1996

US-CL-CURRENT: 435/101, 435/72

ABSTRACT:

A method for causing gelling or increase of viscosity of an aqueous medium containing a gellable polymeric material which has functionalities with phenolic hydroxy groups, and which, in aqueous medium, is susceptible to viscosity increase or gelling in the presence of a carboxylic ester hydrolase, comprises treating the aqueous medium with: a carboxylic ester hydrolase (EC 3.1.1); and an oxidase (EC 1.10.3) and/or a peroxidase (EC 1.11.1); in the presence of an oxidizing agent suitable for use with the oxidase and/or peroxidase. Gelled products obtainable by the method may be dried or dehydrated to give products which are useful as absorbents for absorbing aqueous media, such as body fluids. In particular, gelling a pectic material obtainable from sugar beets using pectinesterase (EC 3.1.1.11) and laccase (EC 1.10.3.2).

16 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (38):

The phenolic-substituted cinnamic acid ester (ferulic acid ester) linkages in phenolic pectins can be hydrolyzed by **ferulic acid esterases**. Enzymes used in the purification of, e.g., polysaccharides containing substituents of the cinnamic acid type should therefore be essentially free from **ferulic acid esterase** activity with specificity towards ferulic acid esters of the polysaccharide in question. Under conditions of low water activity, **ferulic acid esterase** will catalyse the formation of new ester linkages to hydroxyl groups in carbohydrates, and can therefore be used to increase the content of ester residues of the phenolic cinnamic acid ester type (e.g. ferulyl residues) in pectins (including pectin from beet, or from other members of the plant family Chenopodiaceae) and thereby improve their gelling properties in the context of the invention.

Brief Summary Text - BSTX (39):

Thus, under conditions of low water activity, **ferulic acid esterases** may be used to attach groups of the cinnamic acid ester type (e.g. ferulic acid ester groups) to pectins (and possibly other types of, e.g., hydroxylic polymers which are susceptible to viscosity increase or gelling in the presence of a carboxylic ester hydrolase, such as a pectinesterase) which do not contain phenolic residues useful for achieving gelation, and thereby render them susceptible to oxidase- and/or peroxidase-catalyzed gelation.

US-PAT-NO: 5969121

DOCUMENT-IDENTIFIER: US 5969121 A

TITLE: Stable biocatalysts for ester hydrolysis

DATE-ISSUED: October 19, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Allen; Larry	Northfield	IL	N/A	N/A
Aikens; John	LaGrange Park	IL	N/A	N/A
Fonstein; Michael	Chicago	IL	N/A	N/A
Vonstein; Veronika	Chicago	IL	N/A	N/A
Demirjian; David	Chicago	IL	N/A	N/A
Casadaban; Malcolm	Chicago	IL	N/A	N/A

APPL-NO: 08/ 781802

DATE FILED: January 10, 1997

PARENT-CASE:

This application claims priority to U.S. Provisional Application for patent Ser. No. 60/019,580, filed Jun. 12, 1996; Ser. No. 60/009,704, filed Jan. 11, 1996; and is a continuation-in-part of U.S. patent application Ser. No. 08/694,078, filed Aug. 7, 1996, all of which are hereby incorporated by reference in their entirety.

US-CL-CURRENT: 536/23.1, 435/19 , 435/196 , 435/69.1 , 536/23.2

ABSTRACT:

The instant invention encompasses isolated stable esterase enzymes characterized by the ability to remain stable at certain temperatures, substrate specificities, and activity profile.

12 Claims, 121 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 47

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Detailed Description Text - DETX (142):

11. Faulds, C. B. and G. Williamson. (1993) **Ferulic Acid Esterase** from

Aspergillus niger--Purification and Partial Characterization of 2 Forms from a Commercial Source of Pectinase. Biotechnol Appl Biochem. 17:349-359.

US-PAT-NO: 5955137

DOCUMENT-IDENTIFIER: US 5955137 A
See image for Certificate of Correction

TITLE: Ferulic acid decarboxylase

DATE-ISSUED: September 21, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ago; Shoji	Ami-machi	N/A	N/A	JP
Kikuchi; Yasuhiro	Tsukuba	N/A	N/A	JP

APPL-NO: 09/ 018787

DATE FILED: February 4, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	9-025026	February 7, 1997

US-CL-CURRENT: 426/592, 435/161

ABSTRACT:

The present invention relates to a protein having the amino acid sequence represented by SEQ ID NO: 1, or a protein having ferulic acid decarboxylase activity and having an amino acid sequence wherein one or more amino acid residues are deleted, substituted or added in the amino acid sequence represented by SEQ ID NO: 1; a gene encoding said protein; a recombinant vector comprising said gene; a transformant carrying said recombinant vector; a process for producing 4-vinylguaiacol, vanillin or vanillic acid, or a distilled liquor, wherein an enzyme source having ferulic acid decarboxylase activity which is derived from said transformant is used; and a process for producing a distilled liquor, wherein yeast having an enhanced ferulic acid decarboxylase activity is used.

2 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Brief Summary Text - BSTX (8):

It is known that distilled liquors having an excellent flavor can be produced by adding hydroxycinnamic acid ester hydrolase, or a koji mold having a high productivity of hydroxycinnamic acid ester hydrolase, (Japanese Published Unexamined Patent Application No. 115957/95) or **ferulic acid esterase** [Nippon Nogeikagaku Kaishi, 70(6), 684-686 (1996)] to liberate ferulic acid into moromi.

US-PAT-NO: 5882905

DOCUMENT-IDENTIFIER: US 5882905 A

TITLE: Thermostable .alpha.-L-arabinofuranosidase from Aureobasidium pullulans

DATE-ISSUED: March 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Saha; Badal C.	Peoria	IL	N/A	N/A
Bothast; Rodney J.	East Peoria	IL	N/A	N/A

APPL-NO: 08/ 905113

DATE FILED: August 1, 1997

US-CL-CURRENT: 435/105, 435/163 , 435/200 , 435/201 , 435/255.1 , 435/911

ABSTRACT:

An .alpha.-L-arabinofuranosidase enzyme which is highly thermostable, and is effective for the hydrolysis of arabinofuranosyl residues from L-arabinose containing polysaccharides and hemicelluloses is disclosed. The enzyme is produced by color variant Aureobasidium pullulans strain NRRL Y-21792. This .alpha.-L-arabinofuranosidase may be used in conjunction with xylanolytic enzymes for the treatment of hemicellulosic materials to produce fermentable sugars, particularly xylose and L-arabinose.

13 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Detailed Description Text - DETX (13):

Optimal hydrolysis of the hemicellulosic materials to both fermentable sugars L-arabinose and xylose is achieved when the .alpha.-L-arabinofuranosidase is used in combination with one or both endoxylanase and .beta.-xylosidase. Left intact, L-arabinose side chains in hemicelluloses restrict the enzymatic hydrolysis of the substrates by endoxylanase and .beta.-xylosidase. However, treatment of the hemicellulosic materials with the .alpha.-L-arabinofuranosidase cleaves the L-arabinose side groups from the substrate, significantly increasing the subsequent hydrolysis

of the substrate by the endoxylanase and .beta.-xylosidase. Still other xylanolytic enzymes which are effective for removing side groups from polymeric xylans and which may be used herein include **ferulic acid esterase,** .alpha.-glucuronidase, acetyl xylan **esterase, and p-coumaroyl esterase**.

US-PAT-NO: 5872091

DOCUMENT-IDENTIFIER: US 5872091 A

TITLE: Cleaning compositions containing plant cell wall degrading enzymes and their use in cleaning methods

DATE-ISSUED: February 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cuperus; Roelck A.	Amsterdam	N/A	N/A	NL
Herweijer; Margareta A.	Den Haag	N/A	N/A	NL
Van Ooijen; Albert J.J.	Voorburg	N/A	N/A	NL
Van Schouwen; Dick J.	Vlaardingen	N/A	N/A	NL

APPL-NO: 08/ 737970

DATE FILED: January 30, 1997

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	94201741.9	June 17, 1994

PCT-DATA:
APPL-NO: PCT/EP95/02380
DATE-FILED: June 19, 1995
PUB-NO: WO95/35362
PUB-DATE: Dec 28, 1995
371-DATE: Jan 30, 1997
102(E)-DATE:Jan 30, 1997

US-CL-CURRENT: 510/300, 134/42 , 510/320 , 510/321 , 510/392 , 510/393 , 510/530 , 8/137

ABSTRACT:

This invention relates to a laundry detergent composition as well as a method of laundrying fabric or soiled garments in which the composition requires a surfactant, a pectinase enzyme, a specific hemicellulase enzyme or enzymes, a cellulase enzyme, and additional component which includes bleaching agents, builders, an amylase enzyme and/or a protease enzyme.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (26):

Endo- and exo-xylanases and accessory enzymes such as glucuronidases, arabinofuranosidases, acetyl xylan **esterase and ferulic** acid or **coumaric acid esterase** have been summarized by Kormelink (1992, Ph.D.-thesis, University of Wageningen, The Netherlands). They are produced by a wide variety of micro-organisms and have varying temperature and pH optima.

Claims Text - CLTX (4):

C) a hemicellulase enzyme selected from the group consisting of xylanase, arabinofuranosidase, acetyl xylan **esterase, glucuronidase, ferulic acid esterase, coumaric acid esterase,** endo-galactanase, mannanase, lichenase, endo- or exo-arabinanase, exo-galactanase or mixtures thereof;

Claims Text - CLTX (18):

(C) a hemicellulase enzyme selected from the group consisting of xylanase, arabinofuranosidase, acetyl xylan **esterase, glucuronidase, ferulic acid esterase, coumaric acid esterase,** endo-galactanase, mannanase, lichenase, endo- or exo-arabinanase, exo-galactanase, or mixtures thereof;

US-PAT-NO: 5824533

DOCUMENT-IDENTIFIER: US 5824533 A
See image for Certificate of Correction

TITLE: Orpinomyces xylanase proteins and coding sequences

DATE-ISSUED: October 20, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Li; Xin-Liang	Athens	GA	N/A	N/A
Ljungdahl; Lars G.	Athens	GA	N/A	N/A
Chen; Huizhong	Athens	GA	N/A	N/A

APPL-NO: 08/ 445090

DATE FILED: May 19, 1995

US-CL-CURRENT: 435/209, 536/23.2 , 536/23.74

ABSTRACT:

Xylanases having high specific activities from Orpinomyces sp. strain PC-2 are provided as well as methods for their purification. DNA sequences encoding these proteins are also provided.

32 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

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Brief Summary Text - BSTX (6):

Enzymatic conversion of xylan to its monomeric components requires the participation of several enzymes including xylanase (EC3.2.1.8), .beta.-xylosidase (EC3.2.1.37), .alpha.-L-arabinofuranosidase (EC3.2.1.55), .alpha.-glucuronidase (EC3.2.1.1), acetyl xylan esterase (EC3.1.1.6) as well as **p-coumaroyl and feruloyl esterases** [Borneman, W. S. et al. (1993) "Feruloyl and **p-coumaroyl esterases** from the anaerobic fungus Neocallimastix MC-2: Properties and functions in plant cell wall degradation," In: Hemicellulose and Hemicellulases, M. P. Coughlan and G. Hazelwood, Eds. (Portland Press, Cambridge, U.K.) pp. 85-102; Castanares, A. et al. (1992) "Purification and properties of a feruloyl and **p-coumaroyl esterase** from the fungus Penicillium pinophilum," Enzyme Microb. Technol. 14:875-884; Christov, L. P. and Prior,

B. A. (1993) "Esterases of xylan-degrading microorganisms: Production, properties, and significance," Enzyme Microb. Technol. 15:460-475; Eriksson, K.-E. L. et al. (1990) "Microbial and enzymatic degradation of wood and wood components," Springer-Verlag (New York, N.Y.)].

US-PAT-NO: 5591619

DOCUMENT-IDENTIFIER: US 5591619 A
See image for Certificate of Correction

TITLE: Aureobasidium pullulans xylanase, gene and signal sequence

DATE-ISSUED: January 7, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Xin-Liang; Li	Athens	GA	N/A	N/A
Ljungdahl; Lars G.	Athens	GA	N/A	N/A

APPL-NO: 08/ 315695

DATE FILED: September 30, 1994

US-CL-CURRENT: 435/201, 435/254.21 , 435/69.1 , 536/23.2 , 536/23.4

ABSTRACT:

A xylanase from Aureobasidium pullulans having a high specific activity is provided as well as a signal protein for controlling excretion into cell culture medium of proteins to which it is attached. DNA encoding these proteins is also provided.

16 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

---------- KWIC ---------

Brief Summary Text - BSTX (5):

Enzymatic conversion of xylan to its monomeric components requires the participation of several enzymes including xylanase (EC3.2.1.8), .beta.-xylosidase (EC3.2.1.37), .alpha.-L-arabinofuranosidase (EC3.2.1.55), .alpha.-glucuronidase (EC3.2.1.1), acetyl xylan esterase (EC3.1.1.6) as well as **p-coumaroyl and feruloyl esterases** (Borneman, W. S. et al., "Feruloyl and p-**coumaroyl esterases** from the anaerobic fungus Neocallimstix MC-2: Properties and functions in plant cell wall degradation," In: Hemicellulose and Hemicellulases, (M. P. Coughlan and G. Hazelwood, Eds., Portland Press, Cambridge, U.K.) (1993) pp. 85-102; Castanares, A. et al., "Purification and properties of a feruloyl and p-**coumaroyl esterase** from the fungus Penicillium

pinophilum," Enzyme Microb. Technol. (1992) 14:875-884; Christov, L. P. and Prior, B. A., "Esterases of xylan-degrading microorganisms: Production, properties, and significance," Enzyme Microb. Technol. (1993) 15:460-475; Eriksson, K.-E. L. et al., "Microbial and enzymatic degradation of wood and wood components," Springer-Verlag, New York (1990)).

Other Reference Publication - OREF (5):

Borneman, W.S. et al., "Feruloyl and p-**coumaroyl esterases** from the anaerobic fungus Neocallimastix MC-2: Properties and functions in plant cell wall degradation", In: Hemicellulose and Hemicellulases, (M.P. Coughlan and G. Hazelwood, Eds., Portland Press, Cambridge, U.K. (1993) pp. 85-102.